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Microscopic observation of conidia from the genus of *Pleurotus*

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느타리버섯屬에서 Conidia의 顯微鏡的 觀察

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ABSTRACT: Formation of conidium, an asexual organ on the hyphae, was examined from ten species of *Pleurotus*. Conidiospores of them were distinguished into two types of spores; an elliptical and a globose spores. Dikaryotic hyphae of ten species and monokaryotic hyphae of three species were observed to produce conidiospore. Conidia were observed on the hyphae grown on mushroom complete agar medium but were not on mushroom minimal agar medium. Two aconidial mutants were obtained by the ultraviolet irradiation.

KEYWORDS: *Pleurotus*, Conidia formation, Basidiomycotina.

Pleurotus is a popular mushroom due to its delicious taste and good flavor in Korea. As cultivation area of the mushroom is rapidly extended, cultivating species of the mushroom are various; *Pleurotus ostreatus, P. florida* and *P. sajor-caju. Pleurotus genus* is belong to basidiomycetes and is called oyster mushroom that gives lilac to grayish-tinged spore print on white paper. *P. ostreatus* form four terminal basidiospores on the club-shaped and unicellular basidia. Mycelium germinated from a basidiospore is monokaryon with single nucleus. Mycelial anastomosis between compatible two different hyphae can produce dikaryotic mycelia with two nuclei per one cell compartment. Mated heterakaryon was confirmed by the formation of clamp connection which was evidence of that two nuclei present in a cell and of the nuclear migration.

As asexual cycle presented in basidiomycetes, *Flammulina velutipes, Coprinus fimetarius, Pholiota nameko* and *Auricularia auricula* have oidia on the hyphae and *Volvariella volvacea* produced chlamydospore (Raper, 1978).

In *Pleurotus ostreatus,* oidia were only observed on the monokaryotic hyphae (Eugenio, 1968).

However, The conidia were found in dikaryotic hyphae as well as monokaryotic hyphae of the *Pleurotus* in this report. Some conditions affecting conidia formation were also reported.

Materials and Methods

Pleurotus species used in this experiment were described in Table I. Media for mycelial growth to investigate conidia were mushroom complete medium, mushroom minimal medium (Raper, 1972), and McClary's medium containing 1% potassium acetate, 0.25% yeast extract and 0.1% glucose. The species of *Pleurotus* indicated in Table I were grown on potato dextrose agar (PDA) at 25C, stored at 4C and transferred, if necessary. All species were grown in the above medium at 25C for 2 weeks.

The conidia formation was observed under the microscope of Leitz Wetzlar Germany.

Mycelia were placed on the slide glass with one

Table I. Origin of used cultures in *Pleurotus* species.

Spedies	No. of strain	Origin	Date of collection
P. cornucopiae	ASI 2011	Japan	'74. 5.
P. cystidiosus	ASI 2082	Thailand	'82. 9.
P. dryinus	ASI 2123	U.S.A	'86. 1.
P. eryngii	ASI 2125	U.S.A	'86. 1.
P. florida	ASI 2013	Hong Kong	'77. 7.
	ASI. 2015	Germany	'76. 5.
	ASI. 2016	Germany	'76. 6.
P. ostreatus	ASI 2018	Korea	'78. 7.
	ASI 2063	Japan	'79.11.
	ASI 2126	U.S.A.	'86. 1.
P. pulmonarius	ASI 2091	Japan	'81. 8.
P. sajor-caju	ASI 2070	India	'82. 9.
P. salmoneo-stramineus	ASI 2172	Taiwan	'89.10.
P. sapidus	ASI 2124	U.S.A.	'86. 1.

*ASI: Agricultural Science Institute (Korea)

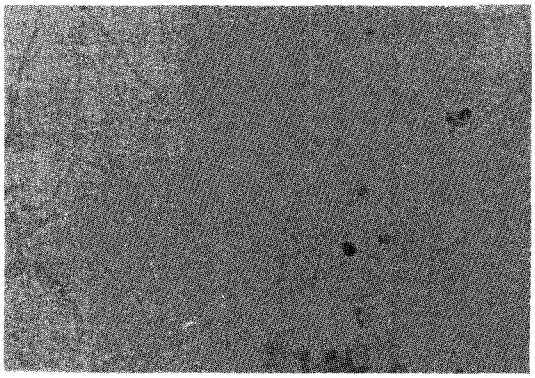

Fig. 1. Conidia from *P. florida* hyphae grown on mushroom complete medium.

drop of 3% KOH solution and covered with cover glass for observation of hyphae. Staining of sample was done with 1% congo red solution and 1% phloxine solution simultaneously and then washed with 3% KOH several times. The drawings were made with a camera lucida.

Mutation was induced using basidiospore suspension of *P. ostreatus, P. florida* and *P. sajor-caju,* and mutagenic treatment was done by ultra violet irradiation as described by Yoo (1985).

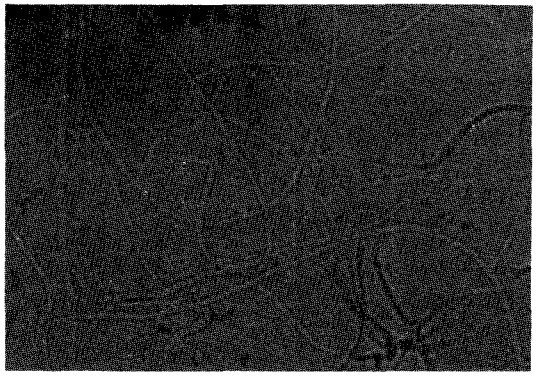

Fig. 2. Elliptical conidia of *P. florida.*

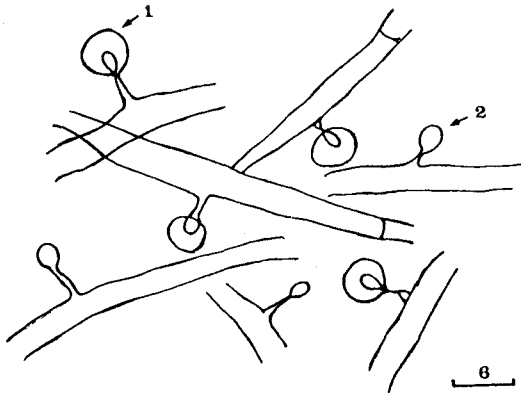

Fig. 3. Globose and elliptical conidia formation on the monokaryotic hyphae of *Pleurotus florida* 1. Globose conidia 2. Elliptical conidia.

Results and Discussion

Ten species of the genus *Pleurotus* collected in Korea or introduced from the foreign countries were grown on mushroom complete agar medium.

When mycelia of the cultures were fully grown on mushroom complete medium for two weeks, hyphae was observed under the microscope.

Hyphae produced conidiophore and a conidiospore. A hyaline conidium was connected with hyphae by the conidiophore. Types of conidia were distinguished into globose and elliptical spores. The size of conidiospore was from 2.4×2.4 μm to 4.8×4.8 μm in globose conidia and from 1.6×2 μm to 2×2.4 μm in elliptical one (Fig. 1-3). Among the ten species tested, all species were observed to produce conidia on hyphae.

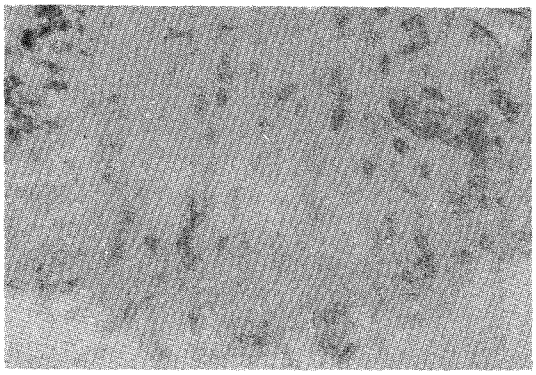

Fig. 4. Conidia on coremia of *P. cystidiosus*.

Pleurotus florida, P. ostreatus, and *P. sapidus* were observed to produce both globose conidia and small elliptical conidia. *P. cornucopiae, P. dryinus, P. eryngii, P. pulmonarius* and *P. sajor-caju* produced only elliptical type of conidia.

Pleurotus cystidiosus formed conidia on black-headed coremia as well as elliptical conidia on hyphae. Conidia on coremia were brown thick wall and slightly curved with rounded ends. However, conidia on hyphae were hyaline round or elliptical compared to the conidia on coremia (Fig. 4).

For effective observation of conidia and hyphae, the hyphae grown on mushroom complete medium was stained using congo red and phloxine solutions mentioned in methods.

Congo red stained the walls of hyphae whereas phloxine was used to stain the interior of the hyphae. In order to improve the contrast of the staining reaction, a few drops of 3% KOH solution were treated and washed several times (Largent 1977).

Globose conidia were distinguished into dark stained inner part and the other outer part of pale color. These two sections were also observed in unstained conidia (Fig. 5).

Monokaryotic hyphae of *Pleurotus* was observed to produce the conidiospores such as dikaryotic hyphae shown in Table II. (Fig. 5).

Conidia formation was variable depending on the culture media. When the mycelia were grown on McClary's sporulation media or mushroom minimal media, they did not produce any conidia

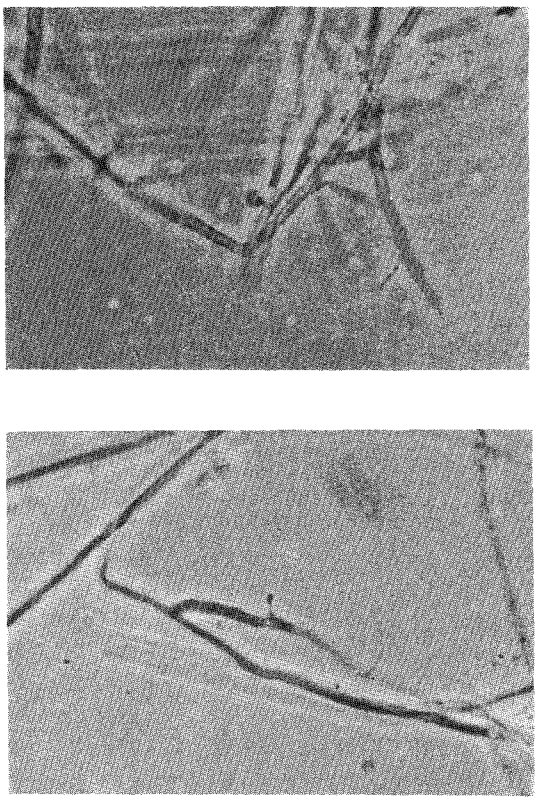

Fig. 5. Conidia on the hyphae of *P. florida* monokaryon after staining with 1% congo red solution (the upper) and 1% phloxine soultion (below).

Table II. Conidia formed on dikaryotic hyphae and coremia in ten *Pleurotus* species.

Species	Globose conidia	Elliptical conidia	Conidia on coremia
P. cornucopiae 2011	−	+	−
P. cystidiosus 2082	−	+	+
P. dryinus 2123	−	+	−
P. eryngii 2125	−	+	−
P. florida 2013, 2015, 2016	+	+	−
P. ostreatus 2018, 2063, 2126	+	+	−
P. pulmonarius 2091	−	+	−
P. sajor-caju 2070	−	+	−
P. salmoneostramineus 2172	−	+	−
P. sapidus 2124	+	+	−

*+: Conidia formation, −: No formation.

Table III. Influence of different media on conidia formation of monokaryotic cultures in *Pleurotus* species.

Monokaryotic culture	Conidia formation*		
	MCM	McClary's medium	MMM
P. florida			
2016-7-37-5	EC	–	–
2016-7-37-23	–	–	–
2016-Ribo	GC		
P. ostreatus			
2018-4	GC		
2054-5	EC, GC		–
2097-60-15	EC, GC	–	

*EC: Elliptical type, GC: Globose type, –: No conidia.

Table IV. Conidia formation of some mutant strains induced by U. V. lights.

Monokaryon strain		Mycelial* growth on MCM	Conidia formation**	
			Globose	Elliptical
P. florida	2016-Ribo	F	++	+
	2016-7-37	M	+	+
	2016-7-37-23	S	–	–
P. ostreatus	2018-4	M	+	+
	2018-Arg	F	–	–
P. sajor-caju	2070-3-56	M	+	+

* Mycelial growth: F; Fast, M;Moderate, S; Slow
**Conidia formation:++, Many; +, A few; –None

(Table III).

However, many chlamydospores were formed on McClary's sporulation media compared to mushroom complete media and mushroom minimal media.

McClary's medium was effective medium for yeast spore formation (McClary, 1959).

In *Neurospora* aerial mycelia are regarded as the first step in conidiogenesis. Aerial mycelia and conidia can be induced by starvation and exposure to light (Clutterbuck 1978). Isocitrate lyase has been shown to increase in specific activity during sporulation in *Aspergillus niger* and *Neurospora.* Isocitrate lyase have been shown to be induced in the acetate, highly conidiating, cultures (Turian, 1972).

In this experiment, hyphae grown on mushroom complete medium, containing aerial mycelia, developed conidia. However, hyphae of some *Pleurotus* species grown on McClary's media containing acetate was observed not to develop aerial mycelia as well as conidia.

Among several mutants obtained from spores of *Pleurotus florida, P. ostreatus* and *P. sajorcaju* by ultra violet irradiation, two strains were observed not to develop any conidia (Table IV). One of the non-conidiating culture, 2016-7-37-23 showed slow mycelial growth and the other culture, 2018-Arg showed normal growth.

Yoo (1985) disclosed that auxotrophic mutants were obtained by ultraviolet irradiation in *P. ostreatus* and *P. florida.*

Mutant of conidial development was obtained with different mutagens such as ultraviolet irradiation, nitrous acid, diethyl sulfate and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) treatment. Several kinds of conidial mutants including aconidial mutant were obtained. Aconidial mutant which is totally lacking conidia was rare in *Aspergillus nidulans* (Clutterbuck, 1969).

In *Neurospora crassa,* some morphological mutants did not produce conidia. A colonial type of non-conidial mutant was abnormal slow-growing mutants characterized by modified cytochrome spectrum (Turian, 1972).

Pleurotus ostreatus has oidia only in monokaryon strain (Eugenio, 1968). Hilber (1982) described that *Pleurotus cornucopiae, P. pulmonarius* and *P. eryngii* have conidia on heterokaryotic mycelia.

This study confirmed that many other *Pleurotus* species beside the species that Hilber investigated produced conidia on their hyphae. Furthermore both monokaryotic and dikaryotic mycelia of the species developed conidia. However, the role of the conidia was not elucidated. It was described that *P. ostreatus, P. cornucopiae* and *P. cystidiosus* attached nematodes and utilized the nutrients in their prey to supplement the low levels of nitrogen available in wood. By the Thorn's investigation (1984), hyphae of *Pleurotus* converged to the mouth of nematode and digested the contents. In

Hohenbuehelia, conidia produced adhesive knobs to attach and digest nematode.

The further studies of conidia in *Pleurotus* were needed to find its roles.

This studies underlined the conidia formation on monokaryotic mycelia as well as dikaryotic mycelia in several species of *Pleurotus.*

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摘 要

국내외에서 수집된 10종의 느타리버섯 균사를 버섯 완전 배지에 배양한 후 균사체에 형성된 分生子(conidia)를 관찰 하였다. 느타리버섯(*P. ostreatus*), 사철느타리버섯(*P. florida*), 노랑느타리버섯(*P. cornucopiae*), 맛느타리버섯(*P. sapidus*), 여름느타리버섯(*P. sajor-caju*), 분홍느타리버섯(*P. salmoneostramineus*) 외에 *P. dryinus, P. eryngii, P. pulmonarius,* 및 *P. cystidiosus* 균사에서 conidia가 형성되었다. Conidia 형태는 구형과 타원형의 2가지였고 2핵 균사체 뿐만 아니라 1핵 균사체에서도 conidia를 형성하였다. Conidia는 버섯 완전 배지에서 관찰되었으나 버섯 최소 배지에서는 관찰하지 못하였으며 담자포자에 UV를 처리하여 conidia를 형성하지 못하는 변이균주를 선발하였다.

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