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ULTRASTRUCTURE OF *NEMATOCTONUS LEIOSPORUS* HYPHAE

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Abstract

The fine structure of the septal pore apparatus of hyphae of *Nematoctonus leiosporus* has the complex structure characteristic of certain Basidiomycetes. The septal swellings are overarched on either side by a dome-shaped, minutely perforated, membraneous pore cap. However, simple septal pores are also observed in the senescent hyphae cultivated in liquid media. In addition to the normal cytoplasmic organellae, the hyphae of the fungus contain prominent autophagic vacuoles which are otherwise rarely reported.

Introduction

Nematoctonus leiosporus, a nematode-destroying fungus, was firstly described by Drechsler in 1943. Hyphae of this fungus are beset with clamp connections long known being characteristic of certain Basidiomycetes. Nevertheless, since the sexual stage of Basidiomycetes has yet not been observed. Consequently, doubt is cast on the solidarity of its phylogenic relationship to other Basidiomycetes. It is awared that one of the additional specific character inherent to certain Basidiomycetes such as Homobasidiomycetidae or in the Tremellales of Heterobasidiomycetidae is the complex septal apparatus. This septal pore structure was termed as dolipore/parenthesome (Moore and McAlear, 1962), or dolipore/pore cap (Bracker and Butler, 1963) which occurs in the base of basidia (Wells, 1964), clamp connections as well as in vegetative hyphae (Jersild *et al.*, 1969). Assuming *N. leiosporus* also pressents such highly specialized septal pore structure, then from the evolutional standpoints, this might be more convincible in sustaining it as a member of Basidiomycetes. Intending to search such information, ultrastructural investigation of the

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hyphae of *N. leiosporus* was reported here; particularly, emphasis was made on the septal structure.

Materials and Methods

For the examination purpose under the transmission electron microscope, *Nematoctonus leiosporus* culture was grown on half-strength cornmeal agar plates. After incubation for 7–10 days at 25°C, small piece of mycelia bearing agar discs were excised, and fixed with a 2.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.0, for 2 hr. Following several rinses in the same buffer, the samples were fixed with 1% chilled OsO_4 for 1 hr prepared in the same buffer. The materials were washed in distilled water twice and then dehydrated in graded alcohol series, and finally embedded in low viscosity resin (Spurr, 1969). Ultrathin sections were cut with a glass knife on a Reichert Om-U2 ultramicrotome and mounted on collodion coated, carbon stabilized grids. The sections were stained with uranyl acetate and lead citrate (Reynold, 1963), and examined with a Zeiss EM 9 electron microscope, operated at 50 KV.

For scanning electron microscopy, specimens were fixed with a 2.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.0, at room temperature for 2 hr. The fixed materials were washed in distilled water twice, and then dehydrated in graded alcohol series. The samples were then dried in a Polaroon E 3000 critical point drying apparatus using liquid carbon dioxide to replace the alcohol (Anderson, 1951). The dried agar blocs were mounted on specimen stubs by means of double-sided cellotypes, coated with gold in Aka-Shi vacuum evaporator, and examined in a Cambridge 600 scanning electron microscope, operated at 25 KV.

Results

In order to illustrate the morphology of the imperfect stage of *Nematoctonus leiosporus* properly, the obclavate conidium and clamp connection of the fungus were photographed through a light microscope and shown in Fig. 1, 2. The fungus usually secrectes a droplet of mucilaginous substance at the conidium apex while it is still retained at its short conidiophore. The minute mass of adhesive substance is used to stick a passing-by nematode. The detail external feature of the clamp connection shows up more clearly under a scanning electron microscope (Fig. 3).

The fine structures of the septum observed in hyphal elements or in clamp connections exhibite the typical complex pore type defined as dolipore by Moore and McAlear (1962). The texature of the septal swelling and cross wall seems to be homogeneous as no deviation could be found when close

examination of this structure was made (Fig. 4–6). The edges of the thickened septa are covered by a dome-shaped, menbraneous pore cap. The pore cap appears to consist of three distinct layers; its thickness is approximately 30 nm, and shows minute size but regularly spaced perforations (ca. 30–36 nm). This character is similar to that of *Coprinus stercoraius* as reported by Ellis *et al.* (1972) (Fig. 4). The pore cap which surrounds the dolipore seems to be continuous with the endoplasmic reticulum (Fig. 5). The thickness of the septal swelling and the diameter of the septal pore is 150–164 nm and 60–90 nm, respectively, measuring from longitudinal and cross sections cut through the near median regios of the septa (Fig. 4–6). The base of the septal swelling is invested by the endoplasmic reticulum viewing from a cross section (Fig. 6). Occasionally, the septal pore is plugged with electron-dense inclusion of unknown nature (Fig. 5). Some distinguishing tubular-vesicular (Fig. 7), lamellar (Fig. 8), or other various types of mesosomes are often associated around the pore cap region. Abundance of simple septa was also present in the senescent hyphae (40-days old) when the fungus was cultivated in liquid media.

Longitudinal, oblique, and cross sections of the hyphae cut through at different planes or at different developing stages are shown in Fig. 9-13. The hyphae have a fibrillar wall varied with thickness depending on the different stage (Fig. 9, 10, 13). The cell membrane is well defined. Sometimes, invagination occurs and elaborates in forming of the lomasome (Fig. 12). The normal cytoplasmic organellae such as nuclei, endoplasmic reticulum, mesosomes, ribosomes, vacuoles, lipid bodies are more or less uniformly distributed throughout the cytoplasm (Fig. 9–13). In addition to these cytoplasmic constituents, large size autophagic vacuoles (Fig. 12, 13), multivesicle bodies (Fig. 10), slender microtubules (Fig. 10, 12), or spindle-like tubules (Fig. 12) are encountered in the cytoplasm. However, the most noticiable feature of the autophagic vacuoles are their internal contents which involve small discrete inclusions, double membrane vesicles, or even a mesosome (Fig. 12, 13).

Discussion

An investigation of the fine structure of vegetative hyphae of *N. leiosporus* revealed different forms of complex membraneous organellae. The reality of these structure, which have been suspected to be artifacts (Bracker, 1967) would seem to have been established through freeze-etching studeis (Griffiths, 1970). Follow the terminology used by Lalonde and Knowles (1975), these structures, based on their morphology, conformation, or location, are termed as lomasomes, lamellar, or tubular-vesicular mesosomes. Structures referred to here as lamellar mesosomes were called complex concentric membranes by

Fig. 1-3. Light and scanning electron micrographs of conidia, conidiophores, and clamp connection of Nematoctonus leiosporus. Fig. 1. Conidium with glutinous droplet. ×1200. Fig. 2. Condium and clamp connection (arrow). ×1200. Fig. 3. Clamp connection. ×10000.

Fig. 4-5. Longitudinal section through clamp connections revealing the septal swelling, perforated septal pore cap, septal pore, cross wall, tubular-vesicular mesosome. Note the continuation of the endoplasmic reticulum and pore cap. Fig. 4. ×63600. Fig. 5. ×60000.

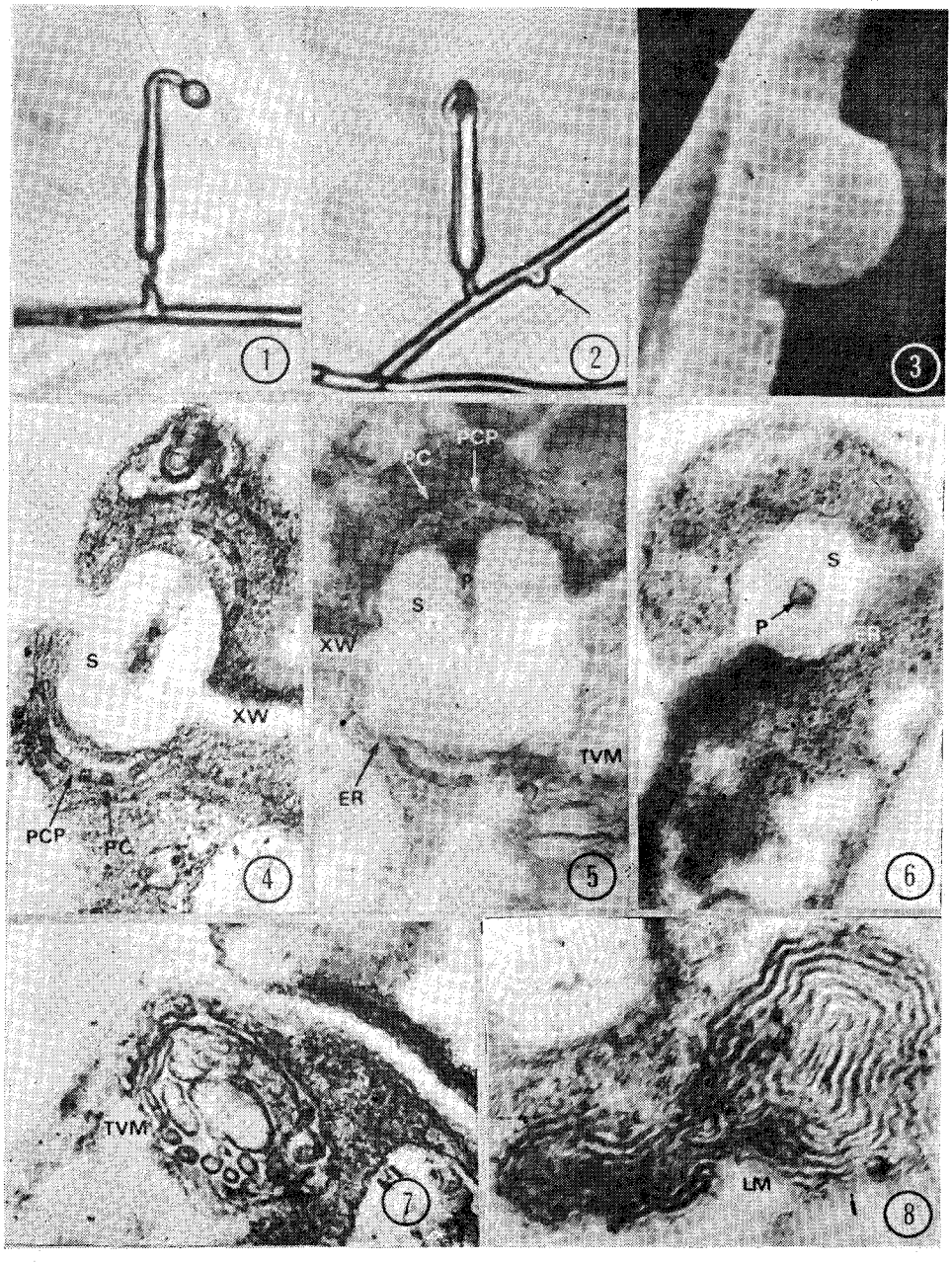
Fig. 6. Cross section through a septal region showing a central septal pore, septal swelling and the surrounding endoplasmic reticulum. ×42000.

Fig. 7-8. Enlargement of tubular-vesicular, and lamellar mesosomes showing different structure. Fig. 7. ×85200. Fig. 8. ×91300.

Fig. 9. Longitudinal section of hyphae of N. leiosporus showing an apical nucleus, electron-dense granules, vacuoles, and bundles of endoplasmic reticulum. 17800.

Fig. 10-11. Oblique sections on hyphae of N. leiosporus illustrating multivesicle bodies, lipid bodies, microtubules, vacuoles, mesosomes, and osmiophilic bodies. Fig. 10. ×41400. Fig. 11. ×23000.

Fig. 12-13. Cross sections of hyphae of N. leiosporus illustrating microtubules, ribosomes, lomasome, autophagic vacuoles, endoplasmic reticulum, mitochondria, fibrillar cell wall, and spindle-like microtubules. Fig. 12. ×40000. Fig. 13. ×31000.
(AV=autophagic vacuole; ER=endoplasmic reticulum; G=electron-dense granule; L=lipid body; LM=lamellar mesosome; LO=lomasome; M=mesosome; MB=multivesicle body; Mi=mitochondrion; MT=microtubule; N=nucleus; OB=osmiophilic body; P=septal pore; PC=pore cap; PCP=pore cap pore; R=ribosome; S=septal swelling; ST=spindle-like tubule; TVM=tubular-vesicular mesosome; V=vacuole; W=cell wall; XW=cross wall)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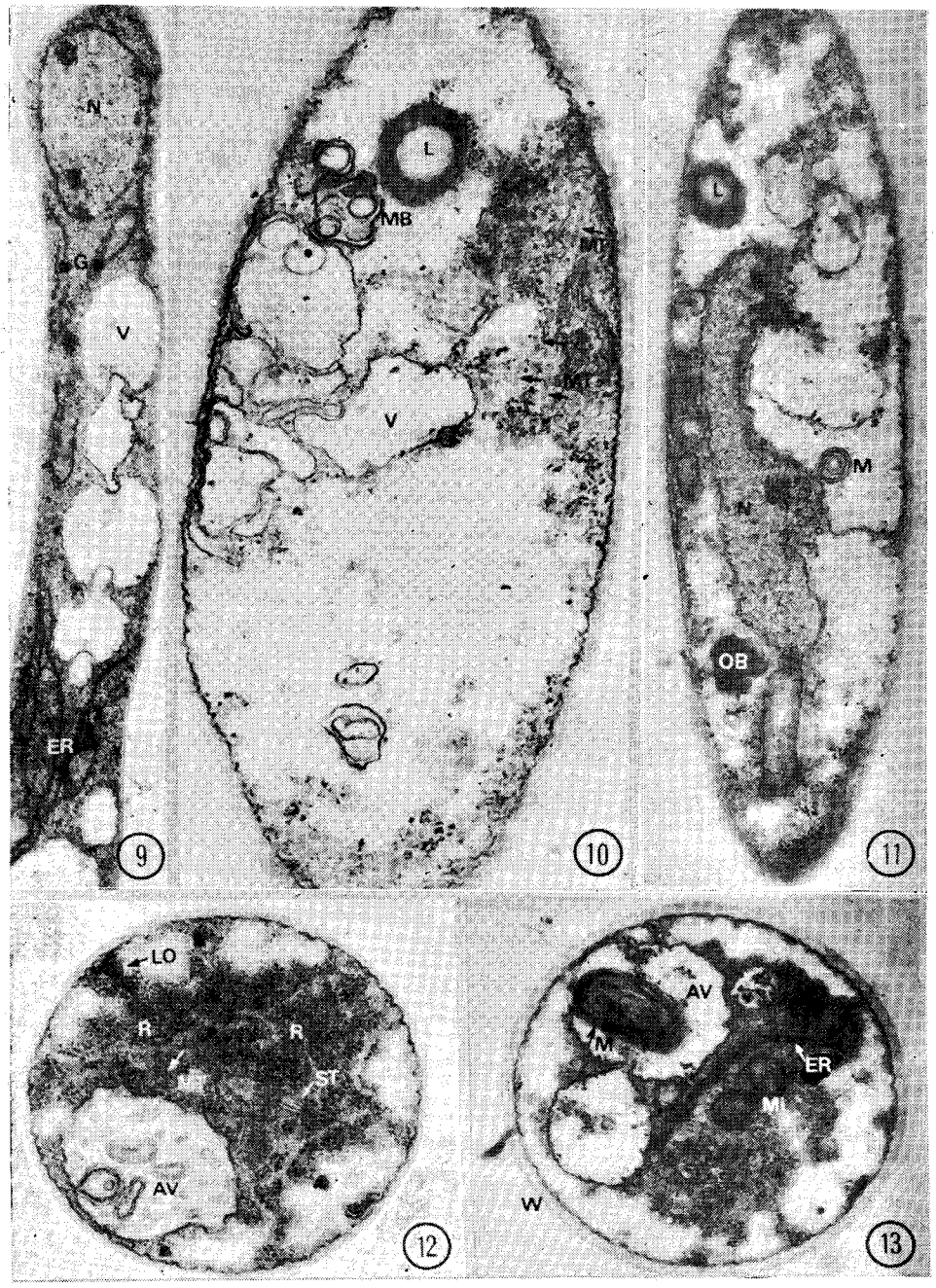
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Hyde and Walkinshaw (1966). They have been considered as components of a convoluted reticulum and responsible for secrection and membrane syntyesis (Berlinder and Duff, 1965). Some tubular-vesiclular mesosomes of *N. leiosporus* are often associated with large lipid bodies. The function of this organella, therefor, is inferred to be related with synthesis or secrection. However, additional functions, such as septal wall formation (Heath and Greenwood, 1970), membrane proliferation (Wells, 1964) can not be ruled out. The multivesicle bodies are probably intimately related to, or even the same entities, as the autophagic vacuoles. The origin of these autophagic vacuoles or multivesicle bodies may be by membrane *de novo* synthesis in the metabolically active sites of the cells (Napolitano, 1963), or by fusion of aged intracellular organellae such as endoplasmic reticulum or mitochondria with the preexisting vacuoles (de Duve, 1963; Novikoff and Essner, 1962). Microtubules, which have been reported in a wide variety of plant and animal cells, and in some bacteria (Newcomb, 1969), were found in *N. leiosporus* when the materials were fixed with glutaraldehyde and osmium tetroxide. This fixation procedure exhibited a well-preserved of the fine structure of the cells than did any of the other procedure tried. The same view-point has been upheld by Hyde and Walkinshaw (1966), and Ellis *et al.* (1972). Nevertheless, whether these microtubules performed a cytoskelton role in the production and maintainance of asymmetric cell division, or aid in the movement of cytoplasmic materials (Newcomb, 1969) in *N. leiosporus* is unknown.

These investigations demonstrated unequivocally that the septa in the hyphae of *N. leiosporus* were different from those of either the Ascomycetes (Moore and McAlear, 1962) or the Deuteromycetes (Reichle and Alexander 1965; Haskins, 1975). They exhibited the typical dolipore/pore cap structures which have been presumed to be characteristic of Basidiomycetes (Moore and McAlear, 1962). The pore cap of *N. leiosporus*, according to the diameter of the pore cap perforations, is of the *Polyporus* type (Wilsenach and Kessel, 1965). This type of pore cap contains small, circular, evenly distributed perforations. In contrast, the pore cap perforations in *Rhizoctonia solani* (Bracker and Butler, 1963), *Exidia nucleata* (Wells, 1964), and *Agaricus campestris* (Manocha, 1965) are large, irregular-shaped, or discontinuous openings. Wilsenach and Kessel (1965) believed the *Polyporus* type to be phylogenically more advanced than the *Rhizoctonia* type, permitting humoral continuity between adjacent cells while maintaining functional diploidy. But this may not the case in *N. leiosporus*, which has not been connected with Basidiomycetes type sexual stage, and heterokarysis has not yet been proved. Bracker and Butler (1963), and Wells (1964) claimed that there was structural and textural difference between the cross wall and septal swelling in *R. solani* and *E.*

nucleata. However, in our micrographs, there is no difference can be readily seen. Close examination of the published electron micrographs of *Coprinus stercoraius* (Ellis *et al.*, 1972), and *Lenzites saepiaria* (Hyde and Walkinshaw, 1966) revealed no difference between the close wall and septal swelling. Accordingly, the diversity is probably due to the different fixation procedure employed or form the different species of fungus investigated. However, the former speculation was further intensified when the papers reported by Manocha (1965), and Berlinder and Duff (1965) were reviewed in detail. Our observations were in agreement with those of numerous workers (Bracker and Butler, 1963; Ellis *et al.*, 1972) in indicating a continuity between septal pore cap and endoplasmic reticulum. The membraneous pore cap may be originated from the modified endoplasmic reticulum (Bracker and Butler, 1963). Simple septal pores have been noticed in aged hyphae of *R. solani* (Bracker and Butler, 1963). The same holds true in *N. leiosporus*. On the contray, simple septa have been obseverd in young hyphae of such Basidiomycetes as *Coprinus lagopus* (compatible mating and common A) (Giesy and Day, 1965), and *Schizophyllum commune* (common A heterokaryon) (Jersild *et al.*, 1967). It was suggested that that the simple septal pore may facilitate nuclei migration between adjacent cells. The mycelium of *N. leiosporus* bears abundant clamp connections and the present investigations confirmed the presence of a dolipore/pore cap structure characteristic of certain Basidiomycetes. It seems more confident to conclude *N. leiosporus* as a member of the Class Basidiomycetes.

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Nematoctonus leiosporus 菌絲之微細構造

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Nematoctonus leiosporus 擁有爲某些擔子菌所特有的，複合的隔膜孔口之微細構造。隔膜之加厚區，兩旁各覆有鐘形，而有規則的微孔之膜質孔蓋。但培養於液體基質的老化菌絲，亦可觀察到簡單的隔膜孔口。除一些常見的細胞胞器外，此眞菌的菌絲亦常含有較少被提及之顯著的自溶液胞。