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TRANSMISSION ELECTRON MICROSCOPE STUDIES OF SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY

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R I N G K A S A N

Pengamatan jamur penyebab penyakit tumbuhan, Sclerotinia sclerotiorum (Lib.) de Bary dengan menggunakan mikroskop elektron telah dikerjakan dalam laporan ini, terutama sekali mengenai sklerotium yang telah masak yang terdiri dari beberapa macam sel yang berbeda-beda. Bagian luar sekali yang merupakan kulit dari pada sklerotium disebut "rind", terdiri dari sel-sel yang berdinding tebal, kaku, kosong dan banyak menerima elektron (electron-dense layer). Macam dinding sel tersebut tidak terdapat pada dinding-dinding sel bagian lainnya. Sedangkan bagian dalamnya yang disebut "medullary hyphae" selalu diselubungi oleh lapisan tebal dan kenyal yang disebut "mucilaginous sheath". Sel-sel tersebut mengandung berbagai bagian seperti inti, mitokondria, benda-benda lemak, benda-benda Woronin, plasma-dalam yang dikelilingi oleh dinding plasma (plasmalemma), retikulum plasma-dalam dan lomasom, seperti pada sel-sel Ascomycetes lainnya.

A B S T R A C T

The ultrastructure of cells of the sclerotia of Sclerotinia sclerotiorum (Lib.) de Bary was studied by electron microscopy. A

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mature sclerotium of S. sclerotiorum contains several types of differentiated cells. The thick-waller rind cells which comprise the sclerotial envelope are empty and have a rough outer electron-dense layer which is absent from the other walls. A mucilaginous sheath was observed around medullary hyphae and the cell contains comprise typical neclei, mitochondria, lipid bodies, Woronin bodies, endoplasm with a surrounding plasmalemma and a sparse endoplasmic reticulum and lomasomes.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary, is a plant pathogen causing serious losses in many agricultural crops both in storage and in the field. It belongs to the Ascomycetes and normally produce sclerotia at some time in its life history. It has also a cross-wall consisting of a simple plate with a central pore, a characteristic which holds also for Deuteromycetes with ascomycetous affinities.

Calonge (1968) observed the ultrastructure of intrahyphal hyphae in *Sclerotinia fructigena*. He also described the multivesicular bodies in the same fungus, together with Fielding and Byrde (1969).

The ultrastructure of the stroma of the brown rot fungi, has also been done in detail by Willetts and Calonge (1969). Moreover, the fine structure of sclerotia in several fungi have also been reported by several authors (Nadakavukaren, 1963; Chet et al., 1969; Nair et al., 1969; Brown and Wyllie, 1970; Wyllie and Brown, 1970).

Jones, in 1970 examined the ultrastructure of sclerotia of *Sclerotinia sclerotiorum*. The results showed that the rind walls of the sclerotia have a rough outer electron-dense layer which is absent from the other walls, and the pseudoparenchymatous walls posses a relatively thin-electron-dense inner wall surrounded by a substantially thicker outer electron-transparent layer.

Maxwell et al. (1972) have also studied the structure of *Sclerotinia sclerotiorum*, on the possible relationships of microbodies and multivesicular bodies to oxalate, endopolygalacturonase, and cellulase (cx) production.

In 1970, Calonge studied the ultrastructure of the microconidium and stroma in *Sclerotinia sclerotiorum*. The results showed thet microconidia posses large nucleus and large lipid body, a few mitochondria and sparse endoplasmic reticulum, whereas stromatal hyphae as food reserve. Moreover, Arimura

and Kihara (1968) have also reported the ultrastructure of this fungus. However, a detail information for this fungus is still need, so in this paper, the ultrastructure of the mature sclerotium of *Sclerotinia sclerotiorum* was reported.

MATERIALS AND METHODS

The culture of *Sclerotinia sclerotiorum* used in this study was originally isolated from diseased french beans. It was grown in Petri dishes, each containing potato dextrose agar medium.

The inoculated agar plates were incubated at 18° - 20° C and the sclerotia which were produced, were collected for observations.

Sample sclerotia were fixed by two methods:

(i) 6% Glutaraldehyde (Sabatini, et al., 1963; Calonge et al., 1969) in 0.1 M phosphate buffer (pH 7.2) for 24 h. at 4°C followed by thorough washing in the same buffer solution, and post-fixation in 2% OsO_4 (in the same buffer) for 4 h. at 4°C.

(ii) 2% $KMnO_4$ unbuffered (Calonge et al., 1969) for 30 min at room temperature, followed by washing in distilled water and staining in 0.5% aqueous uranyl acetate for 3 days at room temperature (Hess, 1966).

The material was dehydrated in a graded ethanol series (50% ethanol plus 0.1% NaCl, 70% ethanol, 95% ethanol, 100% ethanol, for 15 minutes each and then 100% ethanol for 30 minutes) and embedded in Araldite mixture (Calonge et al., 1969).

Sections, 60 - 100 nμ thick, were cut on an LKB ultratome and stained with lead citrate (Mercer and Birbeck, 1961; Reynold, 1963; Jupiter et al., 1970); observations were then made with a Philips Electron Microscope 300.

RESULTS

In most instances, the rind cells of *S. sclerotiorum* were empty or highly vacuolated. Their walls were not abviously thicker (0.25 μ) than those of the cortical hyphae, but the peripheral cells had a thick (1 μ) electron-dense layer outside the cell wall (Fig. 1) and on the outer sclerotial surface the layer was ridges and irregular in appearance. Also, there were discrete electron-dense areas between the other rind cells. Probably these electron-dense areas corresponded to the regions in which melanin pigment had accumulated over

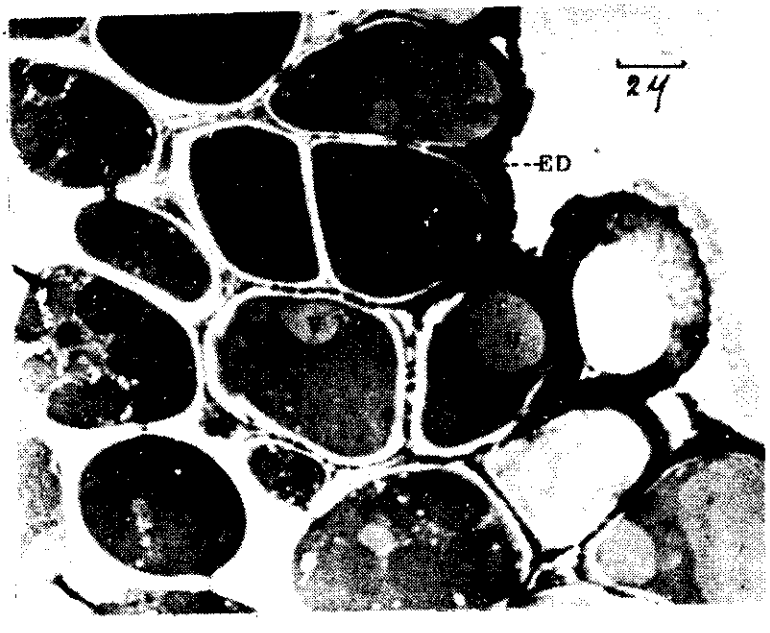

Fig. 1. Electron micrograph of the rind cells of *S. sclerotiorum* showing a thick electron-dense layer outside the cell wall of the rind.

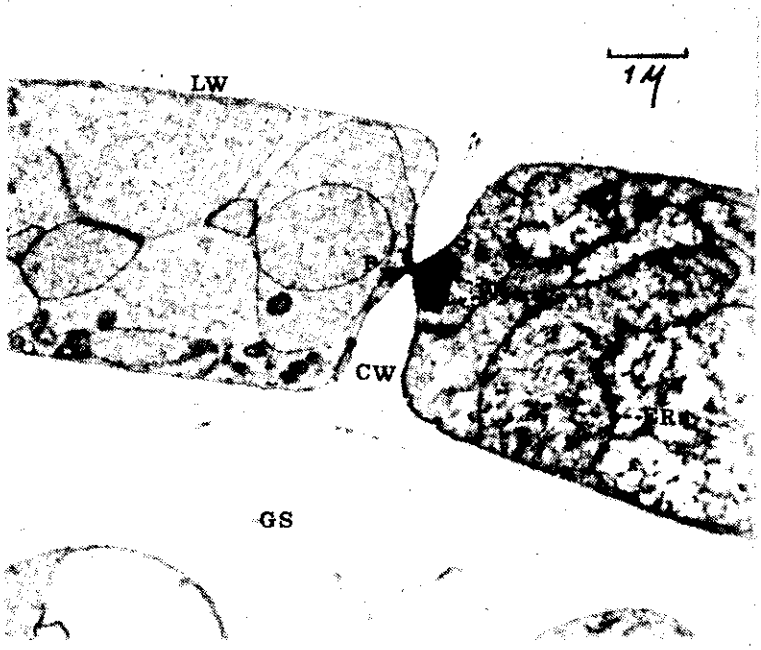

Fig. 2. Longitudinal section of medullary hyphae of *S. sclerotiorum* showing the cross-wall.

and between the cells and served a protective function. Intercellular pigmentation was not apparent in the cortex or medulla. When mature sclerotia were dried, the peripheral cells usually ruptured and this was probably associated with the lack of wall-thickening, the brittleness of the walls and/or the absence of cell contents.

The cortical and medullary hyphae had cell walls approximately 0.36 μ thick; occasionally walls of 0.43 μ thickness were observed. There were no obvious layer in the lateral walls. The cross walls were twice as thick (0.72 μ) as the lateral walls but became narrower towards the simple septal pores (Figs. 2 and 3). Three layers were distinguishable in the septum; a central electron-transparent layer separating two electron-dense layers, similar to that of the lateral walls (Fig. 3).

The cortical and medullary hyphae were similar in most respects except that the former were more closely interwoven and in this investigation their fixation with potassium permanganate was poor. Thus, it was difficult to distinguish membrane systems in them. In the medullary cells the cytoplasmic membrane (plasmalemma) was smooth, in close contact with the cell wall (Fig. 4), and continuous from cell to cell through the septal pore. Numerous investigations of the plasmalemma were observed and these were thought to be lomasomes (Fig. 5). They varied in distribution and appearance; some were granular and others contained small vesicle-like structures. The membrane forming the endoplasmic reticulum was not as thick as the plasmalemma and appeared as two electron-dense layers separated by a wide electron-transparent layer. The endoplasmic reticulum was irregularly distributed in the cells.

In some of the medullary hyphae it was difficult to find nuclei and none were positively identified in cortical cells. Each nucleus observed in the medulla was surrounded by a clearly defined double membrane which was perforated by pores. The nucleoplasm was finely granulated and with a darkly staining nucleolus (Fig. 4).

No mitochondria were distinguished in cortical hyphae under the electron microscope due to poor permanganate fixation, but some were seen in the medulla although they were often difficult to identify. The mitochondria were usually circular or broadly elliptical (Fig. 6) and varied greatly in size (0.96 - 1.65 μ x 2.52 - 3.09 μ) and distribution.

When the sclerotia had been fixed with glutaraldehyde and osmium tetroxide the cortical hyphae were found to be very well-stocked with large electron-dense bodies. These have been interpreted as being lipid droplets and probably serve as reserve materials. In some of the lipid bodies there were very dark areas (osmiophilic globules) which were not identi-

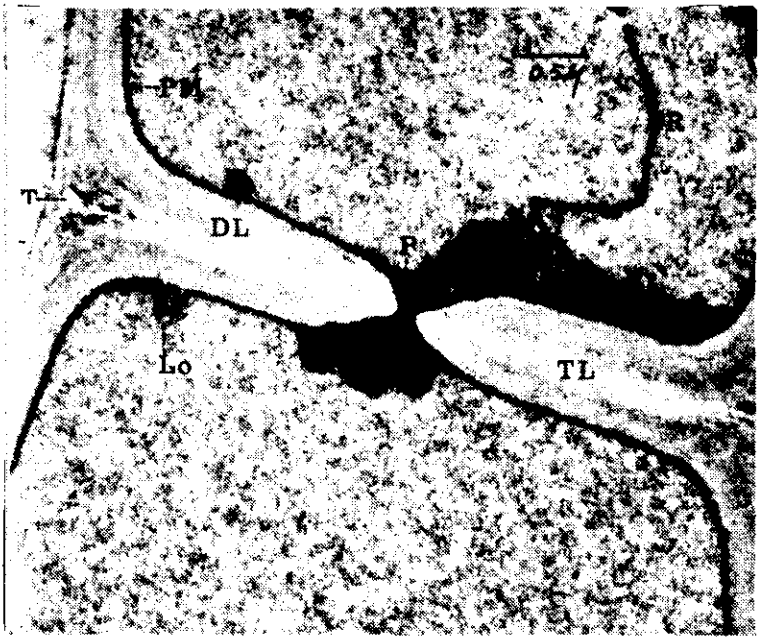

Fig. 3. Longitudinal section of medullary hyphae of *S. sclerotiorum* showing a perforated septum and three Woronin bodies near the septal pore.

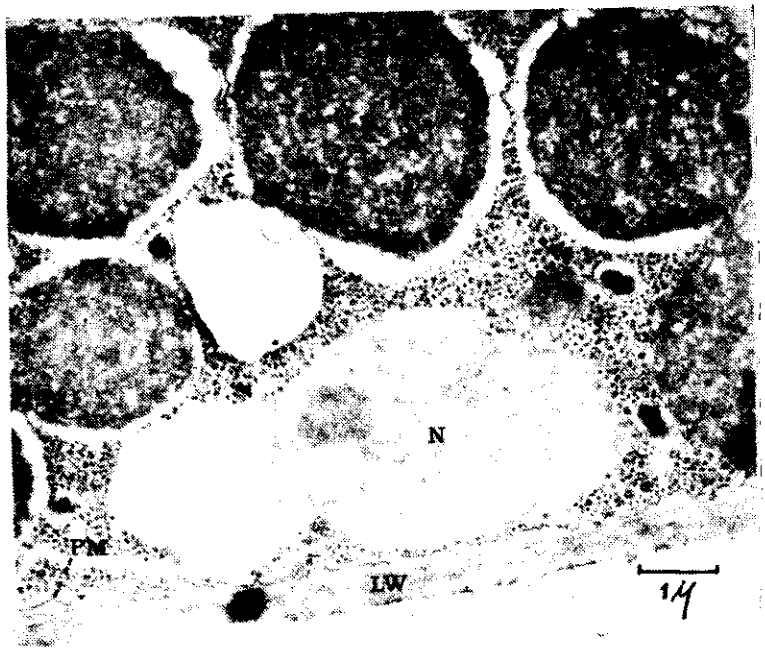

Fig. 4. Section of part of a pseudoparenchymatous hypha showing the nucleus.

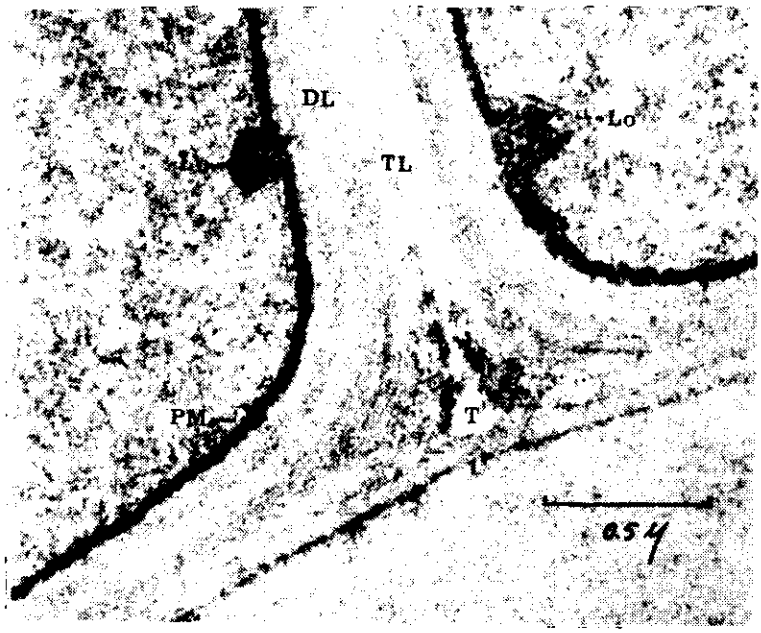

Fig. 5. Ultrathin section through the cross-wall showing the lomasomes.

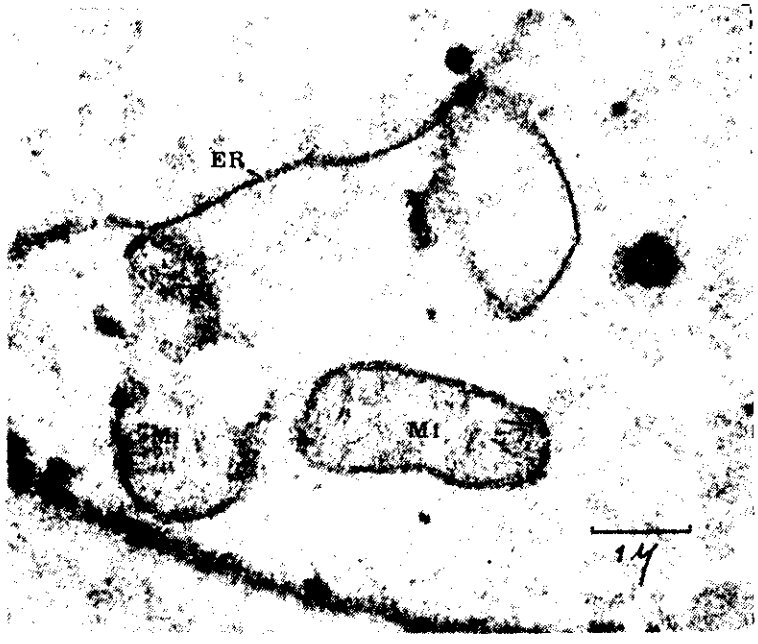

Fig. 6. Section of pseudoparenchymatous hypha showing mitochondria.

fied. Possibly they were lipid droplets in the process of hydrolysis. The distribution of lipid bodies in the medullary cells was uneven with some cells almost filled with them (Fig. 7) and others without any.

It was difficult to identify vacuoles with any degree of certainty. But some of the sclerotial hyphae appear to contain vacuoles, each one surrounded by a single membrane and with electron-dense areas of cytoplasm between the vacuoles. However, the resolution of the membranes in the material examined did not permit a more definite identification.

In many sections, cell inclusions, which were electron-dense and membrane-bound, were observed near septal pores. These structures, after fixation with osmium tetroxide or glutaraldehyde and osmium tetroxide, were the same electron density as lipid bodies and were hexagonal in shape (Fig. 7); when potassium permanganate was used as a fixative, they were oval to spherical in shape and the same electron density as lipid bodies (Figs. 2 and 3). They were always found associated with the septal pore, which they appeared to block, and were considerably smaller (0.32 - 0.49 μ) than most of the lipid bodies (1.80 - 2.88 μ) observed. The former inclusions were in most instances larger than the pores although several small ones were occasionally associated with a single pore. This had been shown in previous report (Kosasih, 1975) and these electron-dense structures were probably Woronin bodies, and were distinguished from lipid bodies by their position in the cell and to some extent, by their smaller size. However, this type of criterion does not give a conclusive identification.

EXPLANATION OF FIGURES

Abbreviation used

CW: Cross-wall,
DL: Electron-dense layer of cross-wall,
ED: Electron-dense area of rind cells,
ER: Endoplasmic reticulum,
GS: Gelatinous sheath,
LB: Lipid body,
Lo: Lomasome,
LW: Lateral wall,
Me: Mesosome,
Mi: Mitochondrion,
N : Nucleus,
P : Septal pore,
PB: Polyhedral body,

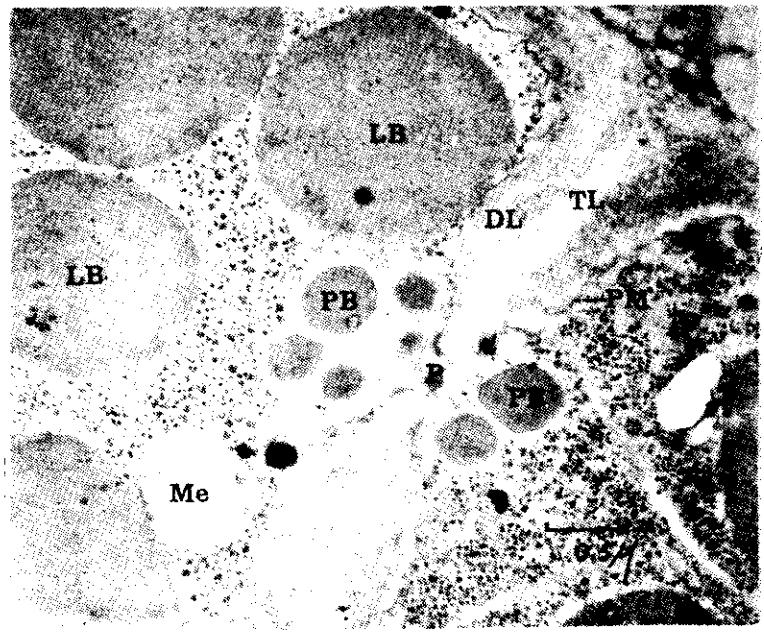

Fig. 7. Ultrathin section of a pseudoparenchymatous hypha of *S. sclerotiorum* showing a perforated septum and polyhedral bodies near the septal pore.

PM: Plasma membrane,
T : Terminal channel,
TL: Electron-transparent of cross-wall,
V : Vacuole,
W : Woronin body.

DISCUSSION

Whetzel (1945) concluded that the sclerotia of *S. sclerotiorum* do not produce mucilage in the medulla but in this investigation a mucilaginous sheath was observed around medullary hyphae when histochemical techniques were used (Kosasih and Willetts, unpublished data). The presence of the sheaths was confirmed by the electron microscope studies. Arimura and Kihara (1968) have also reported the presence of a sheath of mucilage around sclerotial hyphae of *S. sclerotiorum*. Although the mucilage is initially in the form of a sheath, it may be produced in sufficient amounts to fill the interhyphal spaces so that it appears as a mucilaginous matrix in which the medullary hyphae are embedded. Willetts (1972) attributed a morphogenetic function to the mucilage, and probably it also contributes to the resistance of the sclerotium to adverse environmental conditions such as desiccation (Willetts, 1971).

The lateral walls of medullary hyphae consists of a single layer and the cross walls are perforated by a simple pore. The pores were often blocked by small, membrane-bound, electron-dense bodies which were distinct, from the larger, irregularly distributed electron-dense, membrane-bound, lipid bodies observed in storage hyphae. The former were probably Woronin bodies which prevent or regulate the free movement of substances between the medullary hyphae. The conclusion that they are Woronin bodies is based on their size and position in the cell (Kosasih, 1975). Woronin bodies are degraded by pepsin, and lipids are removed from tissues by acetone, but no attempt was made to study the effect of pepsin on the bodies or to use acetone instead of alcohol for the preparation of the fungal material for the electron microscope studies (McKeen, 1971). The large, numerous, lipid globules observed in some cells probably serve as nitrient reserves.

Vacuoles were not positively identified under the electron microscope but they were observed with the light microscope. Probably they are also associated with the storage of food reserves.

The presence of lomasomes has already been observed in a number of fungi (Moore and McAlear, 1961; Wilsenach and

Kessel, 1965; Hendy, 1966; Heath and Greenwood, 1970). It has been suggested that lomasomes have a role in wall synthesis (Bracker, 1976; Willetts and Calonge, 1969).

Some mitochondria were observed in medullary hyphae of mature sclerotia but, probably because of poor fixation, none were distinguishable in cortical hyphae. Wong and Willetts (1974) suggested, from electrophoretic studies of the enzymes at different sclerotial stages, that the glycolytic-Kreb cycle pathway provides energy for the growth of the hyphae at the periphery of the sclerotium but that the phosphate pentose pathway is responsible for supplying the energy for growth and activity of the inner sclerotial hyphae. Histochemical observations indicated that succinic dehydrogenase was very active at the periphery but this was not confirmed by electron microscopy (Kosasih and Willetts, unpublished data). The small number of mitochondria identified can be attributed, partly, to the poor fixation with potassium permanganate as can also the difficulty in detecting nuclei.

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