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ULTRASTRUCTURE OF SEPTA IN *SCLEROTINIA SCLEROTIORUM*

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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 dinding melintang dari pada hypha dan benda-benda sel lainnya yang selalu berhubungan' erat sekali dengan celah di antara dinding melintang, yang disebut "Woronin bodies".

Dinding melintang, terdiri dari tiga lapis, yaitu dua lapis yang banyak menerima elektron (electron-dense layers) dan dibagian tengahnya dipisahkan oleh selapis bagian yang tembus cahaya (electron-transparent central lamella).

Celah pada dinding melintang sering sekali disumbat oleh "Woronin bodies".

A B S T R A C T

An electron microscope study has been done on the structure of septum and its associations of Sclerotinia sclerotiorum (Lib.) de Bary. This septum consists of a cross-wall containing two electron-dense layers separated by an electron-transparent central lamella. The central pore in the cross-wall is partially to completely blocked by cell inclusions called Woronin bodies, which are electron-dense and membrane-bound.

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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 has a cross-wall consisting of a simple plate with a central pore, a characteristic which holds also for Deuteromycetes with ascomycetous affinities.

It is necessary to differentiate between the terms "cross-wall" and "septum". Heretofore the techniques employed to study fungous structures have given rise to concepts which would indicate that the term cross-wall was synonymous with septum. This may still hold true for the Ascomycetes where the septum has no auxiliary structures and appears to be composed entirely of wall material.

Buller (1958) interpreted the septum differently because he viewed it with a phase contrast microscope. The details of the septum clearly lie below the limits of resolution of the light microscope. When an electron microscope is used which has a resolution higher than the light microscope, it is seen that the septum in Ascomycetes is different from the Basidiomycetes in gross structure. It lacks the septal swelling and septal pore cap, which can be distinguished in Basidiomycetes (Bracker and Butler, 1963). According to Buller (1958), in the Ascomycetes the septum consists of a simple disc with a central pore, formed as an annular ingrowth from the lateral wall. This phenomenon agrees with informations of Shatkin and Tatum (1959) and Moore and McAlear (1962).

In 1971, Littlefield and Bracker studied septa in the uredial thallus of *Melampsora lini* (Ehrenb.) Lev. Their results show the same as what was described above for ascomycetous fungi. Similar types of septa has also been observed by Furtado (1971) in the Pyrenomycete *Sordaria fimicola*.

The ultrastructure of *S. sclerotiorum* has also been investigated by Calonge (1970) and Jones (1970), but they did not give detailed information about the septal pore. Therefore, detailed informations of this structure still need to be discussed.

The present investigation was undertaken to describe and discuss the submicroscopic structure of the septal pore in *S. sclerotiorum* (Lib.) de Bary.

MATERIALS AND METHODS

Sample sclerotia of *S. Sclerotiorum* 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 hr 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 hr 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 n μ thick, were cut on an LKB ultratome and stained with lead citrate (Mercer and Birbeck, 1961; Reynolds, 1963; Juniper et al., 1970); observations were then made with a Philips Electron Microscope 300.

RESULTS

A perforated mycelial septum consist of a plate-like cross-wall with a single pore and a characteristic cytoplasmic apparatus in the vicinity of the pore. The cross walls are about 0.72 μ thick at a point about midway between the pore and the longitudinal wall. They are composed of two electron-dense layers separated by an electron-transparent central lamella (Figure 1). The cross walls are thicker near the longitudinal walls and taper to a slightly rounded edge at the margin of the pore. Each electron-dense layer is continuous with the inner portion of the longitudinal wall at the region of the juncture between the longitudinal wall and the cross-wall. The outer portion of the longitudinal wall extends along the long axis of the hypha, even in the region of a septum, and is not invaginated. Occasionally the electron-dense layers of the cross-wall show a lamellar architecture. The plasma membrane is continuous along the faces of the septum and through the septal pore. The central electron-transparent lamella extends from just beside the plasma membrane in the pore to the mid-region of the longitudinal wall. It is not continuous with the outer surface of the hypha. The septal pore (approximately 0.12 μ in diameter) and the pore apparatus are located near the centre of a septum.

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 of the same electron density as lipid bodies and were hexagonal in shape (Figure

2); when potassium permanganate was used as a fixative, they were oval to spherical in shape and have the same electron density as lipid bodies. 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 to the lipid bodies (1.80 - 2.88 μ) observed (Figure 2). The former inclusions were in most instances larger than the pores although several small ones were occasionally associated with a single pore.

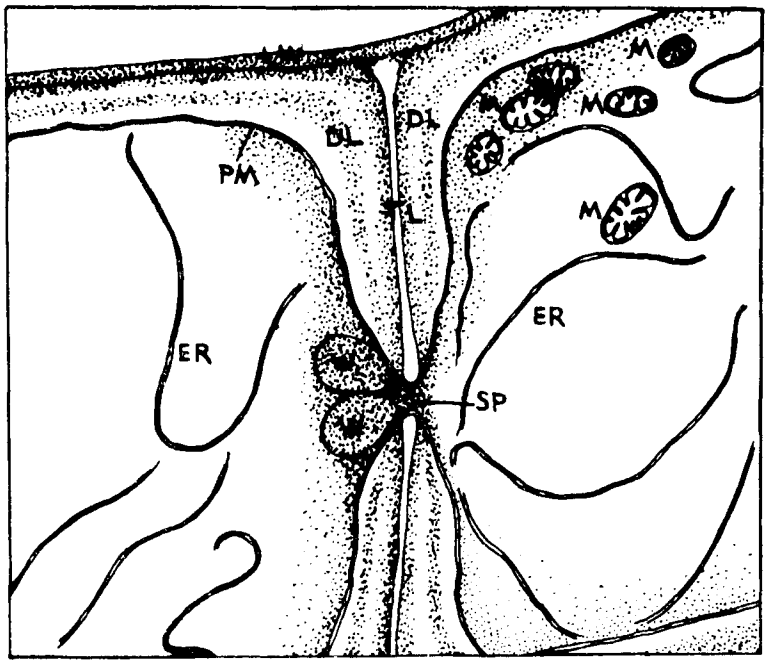

Figure 1

Diagram of fine structure of perforate septum in *Sclerotinia sclerotiorum*

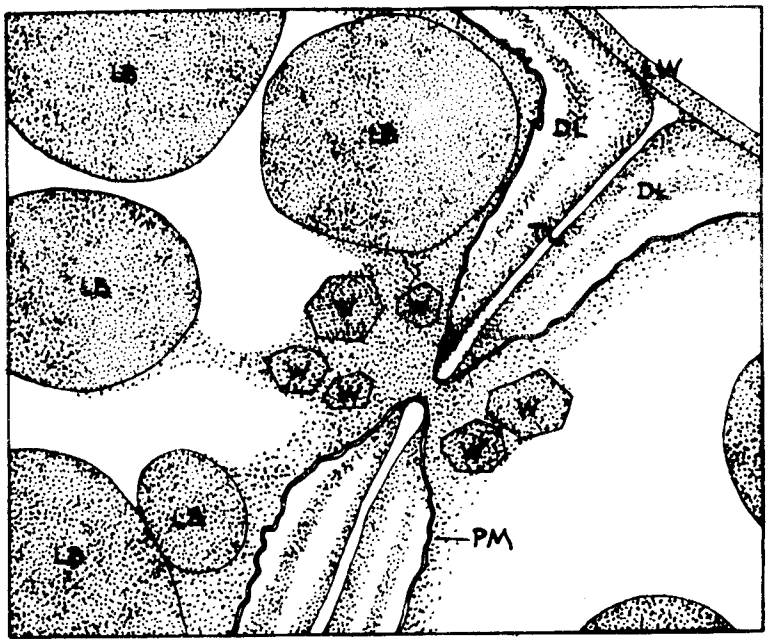

Figure 2

Diagram of fine structure of perforate septum in *Sclerotinia sclerotiorum*, showing the hexagonal shape of Woronin bodies

EXPLANATION OF FIGURES.

Abbreviations used.

DL: electron-dense layer of cross-wall

ER: endoplasmic reticulum

LB: lipid body

LW: lateral wall

M: mitochondrion

PM: plasma membrane

SP: septal pore

TL: electron-transparent layer of cross-wall

W: Woronin body

DISCUSSION

Investigations of fungi with the electron microscope are scarce, and only recently have publications appeared dealing with their ultrastructure.

Septum structure and protoplasmic organization shown with the electron microscope agree with observations on living hyphae within the limits of resolution of the phase contrast microscope. The septum in Ascomycetes is different from Basidiomycetes in gross structure, lacking a septal swelling and septal pore cap.

From our studies on several fungi (unpublished) as well as the observations of others (Bracker, 1967; Willetts and Calonge, 1969; Furtado, 1971), it seems likely that the existence of septal structure similar to that described here will be shown for other Ascomycetes.

The organization of the septal pore apparatus suggests a spacialized structure that is well-adapted for protoplasmic streaming and plugging of the septal pore.

In spite of the simplicity of the ascomycetous septum, there are some reports on elaborate structures associated with the septal pore in certain Ascomycetes (Moore and McAlear, 1962; Reichle and Alexander, 1965; Bracker, 1967).

Our studies on *Sclerotinia sclerotiorum* isolated from different host, show that similarities exist in the kinds of septa present as well as in their structure when compared to other ascomycetous fungi.

The ordinarily perforate septum in filamentous hyphae of *S. sclerotiorum*, consists of a cross-wall containing two electron-dense layers separated by a central electron-transparent lamella. The perforate septum, however, is occluded by electron-dense structures, which may limit intercellular movement of organelles. 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. Similar structures have also been described by Moore and McAlear (1962), Reichle and Alexander (1965), Brenner and Carroll (1968), Carbonell and Rodriguez (1968) and Furtado (1971). Their findings have been equated with the refractile Woronin bodies observed by light microscopy (Buller, 1958). They also described that under the electron microscope, the bodies appear spheroid, electron-dense, and membrane-bound, and some have a crystalline internal structure with a repeating period of about 50 A°.

According to Bracker (1967), Woronin bodies are larger than septal pores and function as pore plugs. Plugging is generally regarded as a protective mechanism, but without some alternative plug type such as protoplasmic sieves, or rever-

sibility of plugging the long-range detrimental effects could outweigh the protective aspects. Further, the organization of the septal pore apparatus suggests a specialized structure that is well adapted for protoplasmic streaming and plugging of septal pore.

The electron micrographs of Moore and McAlear (1962) show that adjacent to the septal pore there are 3 small globules which are smaller than the lipid bodies, but they did not refer to those globules as Woronin bodies. If these bodies plug or seal off the septal pore, the cytoplasmic content of the adjacent cell is disorganized or disappear. This phenomenon has also been observed in the present investigation. Moreover, since these bodies are always in association with the septal pore and agree with the phenomena described above, these bodies seem to be associated with the function of the septal pore and may play a very important part in translocating and limiting the deleterious effects of wounds, or serve to protect a living cell against the effects of the death of an adjacent cell. This fact has also been mentioned by Buller in 1958, using light microscope observations.

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