Electron Microscope Observations of *Helminthosporium victoriae*

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SUMMARY

In this investigation of *Helminthosporium victoriae* routine techniques of electron microscopy were used, except for a slightly modified potassium permanganate fixative. The cell wall consisted of two parts: an outer thin electron-dense layer and an inner broader electron-lucid fibrillar layer. A thin plasma membrane was contiguous with the inner layer of the cell wall. The sparse endoplasmic reticulum ramified throughout the cytoplasm in a sinuous fashion. Mitochondria displayed classical structure and were ubiquitous in distribution. Glycogen and lipids were constantly occurring inclusions. Simple septal pores were present. Large vacuoles with tonoplasts and also numerous smaller vesicles were seen. Hyphae were often multi-nucleate.

INTRODUCTION

A transmissible disease of the fungus *Helminthosporium victoriae* M & M was described by Lindberg (1959) from the cultures obtained from oats with Victoria blight. Light microscope investigations of the normal and the diseased fungus showed that the latter could be distinguished by stunted growth and lysed aerial mycelium (Psarros & Lindberg, 1962). Investigations are now underway to study the pathology of the diseased fungus, and possible identification of the causal agent, by means of electron microscopy. The present paper is concerned with the fine structure of the normal fungus.

METHODS

Vigorous young colonies of *Helminthosporium victoriae* were sectioned into 3 mm. cubes of mycelial mats on agar and transferred to fresh culture plates of potato glucose agar. Small sections were harvested from the corners of the subculture 24–36 hr after plating. These sections contained a small piece of mycelial mat and projecting hyphae 0.5–1 cm. in length. The hyphae were fixed in a 1.5% (w/v) solution of potassium permanganate and a detergent (Werner, Wright & Baker, 1964). Hyphae were rinsed in distilled water, dehydrated in a graded series of ethanol + water mixtures, and embedded in Maraglas (Freeman & Spurlock, 1962). Sections were cut with a diamond knife in a Porter-Blum MT-2 microtome (Ivan Sorvall, Inc., Norwalk, Conn., U.S.A.). All sections were post-stained with lead citrate as described by Reynolds (1963). Photographs were taken with an HU-11A Hitachi electron microscope.

DISCUSSION

The hyphae of *Helminthosporium victoriae* (Pl. 1, figs. 3, 4) were characteristically elongate filamentous structures. The cell walls of *H. victoriae* were considerably different from those of *Rhizopus homothallicus* (Hawker & Abbott, 1963) and *Neurospora crassa* (Shatkin & Tatum, 1959), and thus merit comparisons. The cell wall of *R. homothallicus* consisted of tangentially arranged elongate elements which gave the wall a striate appearance. In *N. crassa* the wall was divisible into two areas. The outer surface consisted of a dense fibriform meshwork. The fibres of this area had a tendency to aggregate and slough-off; consequently this portion of the wall appeared frayed. The inner portion of the wall was a broader zone of low density in which only occasionally could one observe the internal structure which consisted of a network of fine and difficultly discernible fibres. In *H. victoriae* the cell wall (Pl. 1, fig. 1) consisted of an outer thin uniformly electron-dense layer. The frayed appearance of the outer wall, as reported in *N. crassa*, was not evident in *H. victoriae*. The inner wall of *H. victoriae* was invariably broader, slightly fibrillar and quite electron-lucid. The plasma membrane was visible as a thin electron-dense delimiting membrane touching the inner surface of the cell wall (Pl. 1, fig. 1).

Particulate clumped areas in the cytoplasm of *Helminthosporium victoriae* with an electron density greater than that of the cytoplasm were glycogen deposits (Pl. 1, fig. 1). Their size, density, and general appearance agreed with the description of glycogen (Revel, Napolitano & Fawcett, 1960). Lipid inclusions (Pl. 1, fig. 2) were present in most hyphae. Evidently, the lipids in *H. victoriae* contained few unsaturated fatty acids and have reacted to a limited degree, thus leaving the characteristically clear space in the inclusions.

The endoplasmic reticulum (Pl. 1, fig. 2) showed no distinctive pattern, instead ramified throughout the entire cell. This system seemed to occur mostly in a sheet-like form. Serial sections indicated that the endoplasmic reticulum was a relatively sparse organelle.

The hyphae of *H. victoriae* were commonly multinucleated structures. The nuclei had typical double envelopes with intervening pores (Pl. 1, fig. 2). Ordinarily, the nucleoplasm displayed uniform density; however, periodically a considerable amount of electron-density variation was encountered (Pl. 1, fig. 4).

A large vacuole with flocculent contents enclosed by a single-membrane, electron-dense tonoplast was noted (Pl. 1, fig. 4).

Smaller vesicles (Pl. 1, figs. 2, 3) with single-membrane systems and contents with an electron-density similar to that of the cytoplasm were frequently encountered. The vesicle (Pl. 1, fig. 3) might be interpreted either as a plug or as a vesicle about to begin migration through the septal pore. Migration of material and the role of pores were described (Bracker & Butler, 1963; Giesy & Day, 1965).

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EXPLANATION OF PLATE

Helminthosporium victoriae electron micrographs: *cw*, cell wall; *pm*, plasma membrane; *g*, glycogen; *m*, mitochondria; *np*, nuclear pores; *er*, endoplasmic reticulum; *L*, lipid inclusions; *v*, small vesicles; *sp*, septal pore; *n*, nucleus; *va*, large vacuole with tonoplast.

Fig. 1. Cross-section of a vegetative hypha. The cell wall (*cw*) consists of an outer thin electron-dense area and an inner, broader, slightly fibrillar area. The plasma membrane (*pm*) is the single thin electron-dense delimiting membrane. Glycogen (*g*) has a tendency to clump in areas rather than being spread throughout the cytoplasm. Mitochondria (*m*) present the usual picture with double membranes and cristae. Potassium permanganate fixation. $\times 25,000$.

Fig. 2. Cross-section of a hypha. The nuclear membrane is double with typical nuclear pores (*np*). The endoplasmic reticulum (*er*) ramifies irregularly through the cell. Lipid inclusions (*L*) are seen as electron-dense areas and frequently vacuolated. Vesicles (*v*) with single membranes are frequently encountered. Potassium permanganate fixation. $\times 30,000$.

Fig. 3. Longitudinal section through a hypha shows a simple septal pore (*sp*). Vesicle may be a plug or about to begin migration. Potassium permanganate fixation. $\times 12,500$.

Fig. 4. Longitudinal section through a hypha showing the frequent multinucleated condition (*n*). A large vacuole (*va*) surrounded with its tonoplast and enclosed flocculent material is evident. Potassium permanganate fixation. $\times 15,000$.