SHORT COMMUNICATIONS

Fine Structure of Vegetative Hyphae of *Aspergillus fumigatus*

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(Accepted for publication 9 November 1970)

The electron microscopic features of representative species of most of the major fungal groups have now been described. The reviews of Hawker (1965) and Bracker (1967) provide a framework of comparative cytology which is of use in studying additional fungal species.

Ultrastructural studies among the aspergilli have been mostly conducted with *Aspergillus niger*, though Moore & McAlear (1962) included *A. varicolor* in their study of septal pores. The vegetative hyphae of *A. niger* were studied by Tanaka & Yanagita (1963*a*) and by Tsukahara & Yamada (1965). Tanaka & Yanagita (1963*b*) and Winner (1966) have also reported on the fine structure of the conidiophores of this species. The electron microscopy of conidial germination in *A. niger* was reported by Kawakami (1960), Tanaka & Yanagita (1963*a*) and Hawker (1966). A more detailed account was given by Tsukahara (1968).

In view of the medical importance of *Aspergillus fumigatus* Fres. it was considered worthwhile to investigate the ultrastructure of this species in culture to allow comparisons to be made with its appearance in diseased tissues. This paper describes the electron microscopy of thin sections of vegetative hyphae of *A. fumigatus*.

METHODS

The strain of *Aspergillus fumigatus* used in these studies was designated T30e/1, isolated from the infected lung of a gentoo penguin.

Cultures were grown and fixed in two ways. (a) Inoculations were made onto millipore filters on the surface of malt agar plates and incubated at 40° for 18 h. Each filter, with its colony, was then stripped from the agar and KMnO₄ (2 % aqueous, unbuffered) was drawn up through the filter by suction. This procedure enabled thorough wetting of an otherwise hydrophobic colony. Once wetted, the material in fixative was transferred to a refrigerator and fixation continued for 30 min. at 4°. The colony was then gently lifted from the filter and washed in a centrifuge tube containing water. (b) Petri dishes containing glucose+peptone broth about 3 mm. deep were inoculated and incubated at 40° for 18 h. The cultures were then gently centrifuged and re-suspended in a compound fixative (P.G.A.O.) prepared as follows. An 8 % (w/v) solution of *p*-formaldehyde was heated to 90° and the resulting suspension re-dissolved by adding 0.1 N-NaOH drop by drop; 25 ml. of this was then mixed with 5 ml. of 25 % glutaraldehyde and 0.6 ml. acrolein. This fixative was diluted 1:1

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with 0.2 M-phosphate buffer (pH 7.4). Fixation (2 h. at 4°) was followed by three washes of 0.1 M-phosphate buffer and postfixation in 1 % osmic acid (in the same buffer) for 2 h. at room temperature. Material was dehydrated in an alcohol-water series followed by propylene oxide and embedded in Epon. Sections were cut on a LKB Ultratome, stained 15 min. in each of uranyl acetate (satd. aqueous) and lead citrate (Reynolds, 1963) and examined with an AEI EM6B electron microscope.

RESULTS

With the fixatives used there was no evidence of a multilayered hyphal wall, but there was frequently a diffuse flocculent material on the outer wall surface in KMnO_4 fixed hyphae (Pl. 1, fig. 2, arrow) and this may be represented in P.G.A.O. fixations by small dense aggregations (Pl. 1, fig. 3, arrow). The walls varied in thickness from 0.05 to 0.30 μm . but were of uniform thickness locally. Septa were of the ascomycete type with a single central pore. The general features of the cytoplasmic constituents were typical of those described for other species of fungi. Nuclei (Pl. 1, fig. 1) ranged in size from 1.3 to 2.3 μm . $\times$ 0.7 to 1.5 μm . though these may not be median diameters. The overall width of the nuclear envelope was between 0.02 and 0.03 μm ., and the P.G.A.O. fixation rendered it more wrinkled than did permanganate fixation. Typical nuclear pores were common, and occasionally much wider discontinuities in the nuclear envelope were seen. The P.G.A.O. fixation showed a nucleolar mass within each nucleus (Pl. 1, fig. 1).

Mitochondria were extremely variable in shape and size. Among the diverse forms seen, some were elongated, branching or constricted and others were roughly polygonal in shape (Pl. 1, fig. 1) with cristae inserted at the corners. The latter type were common in P.G.A.O. fixed material but were not seen with KMnO_4 fixation.

Other membranous constituents included a scanty endoplasmic reticulum (Pl. 1, fig. 1, 2), subcircular vesicles with homogeneous contents of unknown function (Pl. 1, fig. 2) and multivesicular bodies (Pl. 1, fig. 1, 2) similar to those described in other fungi. The only type of lomasome encountered was apparently similar in structure to the plasma membrane, and no evidence of the type of lomasome possibly derived from multivesicular bodies (Marchant, Peat & Banbury, 1967) could be found.

Some of the cell vacuoles contained tubular structures of indeterminate length and about 0.05 μm . diameter (Pl. 1, fig. 4). The nature of these structures is unknown. Plate 1, fig. 4, also shows a myelin-like membranous structure apparently being released into the vacuole. Ribosomes seen by P.G.A.O. fixation (Pl. 1, fig. 1, 3) were mostly between 0.015 and 0.03 μm . diameter. Some sections showed fewer granules than did others, and this may reflect differences in metabolic activity of the hyphae.

Lipid droplets (Pl. 1, fig. 2) were the most frequently encountered storage bodies, but the P.G.A.O. fixation also showed numerous clusters of electron-transparent granules (Pl. 1, fig. 3), 0.06 to 0.2 μm . diameter, which had diffuse, non-membranous boundaries.

Hyphae with degenerate cytoplasm were seen with both fixation methods. The cytoplasm was contracted away from the wall and contained fragments of membranes, vesicles and other unidentifiable material (Pl. 1, fig. 6). Several instances of intrahyphal hyphae were also seen, in which a degenerate hypha had been colonized.

DISCUSSION

In many respects the structure of vegetative hyphae of *Aspergillus fumigatus* resembles that of other fungi (Hawker, 1965; Bracker, 1967). Although the present work has provided little new information applicable to fungi as a whole, it supports previous findings in other species as well as providing a comparison for studies into the ultrastructure of infections caused by *A. fumigatus*.

The simple septal pore is of the type found in most Ascomycetes and Deuteromycetes, but Tanaka & Yanagita (1963*a*) state that in *Aspergillus niger* they found no evidence of pores other than narrow channels 0.08 μm . diam. The septal pore of *A. niger* was described by Tsukahara & Yamada (1965) as an electron-transparent area in the centre of the septum.

The extreme variation in shape of fungal organelles such as nuclei and mitochondria has been noticed by many workers (e.g. Kawakami, 1961; Hawker & Abbott, 1963; Hawker & Hendy, 1963; Tanaka & Yanagita, 1963*a*; Marchant *et al.* 1967; Prusso & Wells, 1967). The polygonal shape of mitochondria reported here is possibly an artefact of P.G.A.O. fixation, since they were not seen in permanganate-fixed hyphae and are apparently not reported by other workers. The type of lomasome (plasmalemmasomes) which are composed of membranes similar to the cell membrane have also been considered as artefacts (Campbell, 1968). The nature of the myelin-like formations such as those seen in the present work has been debated for some years (Blondel & Turian, 1960; Smith & Marchant, 1967). It is perhaps significant that one such structure was seen apparently entering a cell vacuole, since Smith & Marchant (1967) associated these bodies inside vacuoles with discharge into the vacuoles of amorphous electron-opaque material from cytoplasmic vesicles.

The ribosomes seen were typical of fungi in lying free in the cytoplasm. Their size, 0.015 to 0.03 μm ., compares with 0.012 to 0.015 μm . for *Neurospora crassa* (Zalokar, 1961). The significance of this difference is doubtful, since Koehler (1962) reported variation in ribosomal size with different metabolic states of the cells.

By analogy with other fungi, the electron-transparent granules seen in *Aspergillus fumigatus* are probably storage bodies composed of some polysaccharide (Hyde & Walkinshaw, 1966; Campbell, 1968). No evidence of the type of granules seen in *A. niger* (Tsukahara & Yamada, 1965) has been found in the present work.

The empty degenerate hyphae may be taken as evidence that even when growth is apparently vigorous some hyphal autolysis occurs, and the presence of intrahyphal hyphae supports this (Calonge, 1968).

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EXPLANATION OF PLATE

Key to abbreviations: *er*, endoplasmic reticulum; *g*, granules; *l*, lipid body; *lm*, laminated membranes; *m*, mitochondrion; *m vb*, multivesicular body; *n*, nucleus; *no*, nucleolus; *r*, ribosomes; *t*, tubule; *va*, vacuole; *ve*, vesicle; *w*, hyphal wall. Scale marker = 1 μ m unless otherwise stated below.

Fig. 1. P.G.A.O. fixation.

Fig. 2. KMnO₄ fixation.

Fig. 3. P.G.A.O. fixation.

Fig. 4. P.G.A.O. fixation. Vacuole with inclusions. Scale marker = 0.5 μ m.

Fig. 5. P.G.A.O. fixation. Lomasomes. Scale marker = 0.1 μ m.

Fig. 6. KMnO₄ fixation. Degenerate cytoplasm. Scale marker = 0.5 μ m.