Ultrastructural Observations of Mature and Encysting Zoospores of *Pythium proliferum* de Bary

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Summary

The process of zoospore maturation and encystment in *P. proliferum* was studied by electron microscopy. General ultrastructural features of the mature, swimming zoospore were found to be similar to those previously described for other oomycetes in both the attachment and ultrastructure of the flagella as well as the type and distribution of cellular organelles. Associated with extensive areas of RER in the mature zoospores were unusual, electron-dense, bar-like structures. These structures were found in the groove region of young zoospores and at the periphery of encysting zoospores. Their possible function is discussed. The five main types of vesicles observed during encystment, as seen grouped in this study, along with the vesicles described in previous studies of oomycete encystment, were in table form and individually discussed. Interesting correlations appear to exist in the types of vesicles that are present within the oomycetes studied thusfar.

1. Introduction

In recent years, much information has been reported concerning the general ultrastructural features of the biflagellate, oomycetous zoospore (GAY and GREENWOOD 1966, HO, ZACHARIAH, and HICKMAN 1968, REICHE 1969, GAY, GREENWOOD, and HEATH 1971, GROVE 1971, HOCH and MITCHELL 1972 a, BLAND and AMERSON 1973, GOTTELLI 1974, KOBAYASHI and AKAI 1974 a). Studies involving ultrastructural changes during encystment of such zoospores have, however, been restricted to the genera, *Aphanomyces* (HOCH and MITCHELL 1972 b), *Phytophthora* (HEMMES and HOHL 1971, TOKUNAGA and BARTNICKI-GARCIA 1971, BIMPONG and HICKMAN 1975, SING and BARTNICKI-GARCIA 1975) and *Pythium* (GROVE 1971, KOBAYASHI and AKAI 1974 b). Despite the valuable information obtained from these studies, it is still difficult to correlate their findings because of variations in fixation procedures and because of different interpretations concerning the nature of the numerous vesicular inclusions found in the encysting zoospore. Specifically, many un-
answered questions remain concerning vesicle morphology and cell wall synthesis. The purpose of the present paper, therefore, is to provide further information concerning secondary zoospore ultrastructure and encystment as seen in *Pythium proliferum* de Bary. An attempt has been made also to organize (Table 1) and generally correlate the major vesicle types described previously, and herein, in mature and encysting oomycetous zoospores.

2. Materials and Methods

Cultures of *P. proliferum* were induced to sporulate as described previously (LUNNEY and BLAND 1976). Encystment was achieved by the addition of potato dextrose broth (Difco Laboratories) or olive oil to zoospore suspensions. Both swimming and encysted zoospores were fixed by mixing the water containing the spores with an equal volume of 6% glutaraldehyde that was buffered with 0.2 M sodium cacodylate at pH 7.5. Fixation for 12–15 minutes at room temperature was followed by a 1–2 hours wash in 0.1 M sodium cacodylate. Specimens were then post-fixed in sodium cacodylate-buffered 2% OsO₄ for 10 minutes, dehydrated in a graded ethanol series, and embedded in Araldite 6005 (Fullam, Inc.) or Spurr (Polysciences, Inc.) medium. Zoospores were collected by centrifugation for each solution change. Sections were double-stained with lead and uranium salts and examined with a Hitachi HS-8 or a Philips 201 electron microscope.

3. Results

3.1. General Morphology

Swimming zoospores of *P. proliferum* exhibit the same general morphological features as those described by Ho, HICKMAN, and TELFORD (1968) for the secondary zoospore of several other oomycetes. They are typically ovoid with a deep longitudinal groove (Figs. 1 and 11). Viewed on end, they appear reniform (Fig. 1). Two flagella (anterior tinsel, posterior whiplash) are attached at a protuberance within the groove region (Fig. 6). After swimming for several minutes to several hours, depending on environmental conditions, the zoospores begin to move slowly and in a jerking manner until they finally settle, become spherical in shape, retract or cast off their flagella, and

Fig. 1. Newly-cleaved zoospore of *P. proliferum* still within the sporangial vesicle. Note pyriform nucleus (*N*) with associated dictyosomes (*D*), mitochondria (*M*), and kinetosome (*K*). Vesicular inclusions include peripheral vesicles (*Pe*), lipid (*L*), phospholipid vesicles (*Ph*), and cell wall vesicles (*CW*). Unlabeled arrows show the external limits of the groove region (*GR*). Scale represents 1 μm

Fig. 2. Dictyosome-derived, bristle-coated vesicles found in the groove region. Scale represents 0.5 μm

Figs. 3–5. Mastigoneme formation and release from zoospore. Fig. 3. Longitudinal section through a mastigoneme packet (*Ma*). Fig. 4. Cross section of mastigoneme packet showing its relationship to the perinuclear continuum (*Pc*). Fig. 5. Release of mastigonemes in groove region of the zoospore. Scale represent 0.5 μm