Coelomomyces psorophorae var tasmaniensis Couch + Laird (1988) (Coelomomycetaceae: Blastocladiales), a fungal pathogen of the mosquito Aedes australis
II: Nuclear changes during meiospore formation

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Abstract

The presence of synaptonemal complexes were checked in dividing chromosomes as evidence for meiotic division in germinating sporangia. Continuous urografin gradients were used to separate out the various phases of germinating sporangia, the nuclei were removed and embedded for ultrastructural studies. Meiotic inhibitors were applied to germinating sporangia to retard meiotic division to highlight the synaptonemal complexes. At an early phase of sporangial differentiation dividing nuclei developed with synaptonemal complexes. Meiotic inhibitors and stimulators may be used to control sporangial germination for an induction of a high meiospore count. This may be of crucial importance in the utilization of Coelomomyces spp. as a biological control agent of mosquito species.

Introduction

Coelomomyces spp. are obligate fungal parasites of mosquito larvae. They have a biphasic life cycle, a gametophytic stage in an intermediate crustacean host alternating with sporophytic development in mosquito larva. Coelomomyces spp. have been widely reported from various mosquito species [1] and are the only biological agents known to cause large scale epizootics in mosquito larval populations. They are thought to have considerable potential as biocontrol agents. The survival of the parasite during drought or in the absence of the host mosquito species is due to the presence of so called 'resistant sporangia'. In some species, the sporangia have thick outer walls, in other species there is no such difference. Coelomomyces psorophorae var tasmaniensis Couch and Laird is a New Zealand isolate and is adapted to a brackish water environment. Tenci [2] suggested that there is an inbuilt physiological variation in C. p. var tas. which responds to favourable environmental factors. When ecological conditions are favourable approximately 30% of sporangia are activated at any one time, this results in a staggered germination response, i.e. release of meiospores. A maximised coordinated germination of the bulk of the previous season's (or cycle) sporangia in a natural habitat (such as a newly flooded rice-field) should increase the concentration of germinating meiospores with a consequent effect on mosquito larval populations. The morphological transformations that accompany the germination of sporangia are also
of considerable interest. Whisler et al. [3] confirmed that meiosis occurs in the sporangia of *Coelomomyces psorophorae* during the germination process. The exact timing and details of the process have not, however, been established. In this study, using the synaptonemal complex as a physical marker, we have been able to determine the exact timing of meiotic division in *C. p. var tas*. Synaptonemal complexes, are known to occur only during meiotic division, when the homologous chromosomes (one from each parent) form a bivalent [4]. It is the ‘scaffolding’ of proteinaceous material between these homologous chromosomes, which constitutes the synaptonemal complex. Synaptonemal complexes are made up of two lateral components and a central component – all of which can be confirmed by TEM.

In the previous paper (Buchanan & Pillai, 1990a) we described the changes that occur in the sporangia wall of *C. p. var tas* during germination. In this paper we are reporting on the events leading to the formation of meiospores and their subsequent development prior to release from the sporangia.

**Materials and methods**

Field collected *Coelomomyces* spp. infected mosquito larva when moribund were dissected and the sporangia harvested, washed clear of debris and stored. Sporangia were then activated, fixed and the sporangial stages separated using urografin gradients. Some of the separated sporangial stages were embedded in resin (Buchanan & Pillai, 1990a), and others were fractured and the nuclei separated and embedded.

**Use of meiotic inhibitors**

*During active germination.* Stored sporangia were germinated in a low salt medium as in Buchanan and Pillai (1990a) except the meiotic inhibitors Vinblasticine and Colchicine were included at 5 or 50 μg ml, plus controls with dimethyl sulfoxide (the solvent for Cholchicine and Vinblastine) in a medium suitable for activating *Coelomomyces* sporangia described earlier by Whisler et al. [5]. Germination was halted after 2½ and 5 hours when sporangia were fixed in 2% glutaraldehyde in 0.05 M KH₂PO₄ buffer, pH = 7.0, 22 °C for at least 2 hours. The fixed sporangial population consisted of various stages of germination, these stages were separated on urografin gradients as described earlier by Buchanan & Pillai (1990a).

*After urografin separation.* Stored sporangia were germinated in a low salt activation medium. The germination was temporarily halted by cooling at 4 °C. The sporangial stages were separated on urografin at 4 °C, the bands removed, and then germination was allowed to continue in the presence of Cholchicine or Vinblastine for another 60 minutes at room temperature before glutaraldehyde fixation.

**Separation and purification of nuclei**

The separated sporangia were pelleted in a bench centrifuge (Wifug) speed two (3700 rpm), washed in 0.25 M sucrose, glass beaded [6] in 0.25 M sucrose for one minute (glass beads 1–1.2 mm), put through a rotating homogeniser, sieved (pore diameter 0.3 μm) and centrifuged twice in a Sorvell SE12 rotor. First at 300 g for 15 minutes, the supernant was then spun at 600 g for 15 minutes to pellet nuclei. The nuclei were resuspended in 100–150 μl of 0.25 M sucrose and treated with zymolase for two hours at 37 °C in a water bath. The optimal zymolase concentration (10 units) was obtained by titration against *Saccharomyces cerevisiae* (data not shown). One hundred microliters of 1% trypsin EDTA was then added for 30 minutes at 37 °C in a water bath. The nuclei were pelleted at 600 g, resuspended in 0.25 M sucrose and filtered through 5 μm Millipore filters. The fluorescent nuclear stain – acridine orange along with phase microscopy were used to confirm the presence of nuclei.