

A study of the bryozoan spore stages of *Tetracapsuloides bryosalmonae*, the causative agent of salmonid proliferative kidney disease, using confocal microscopy techniques



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Abstract

Spores of *Tetracapsuloides bryosalmonae* were collected from infected bryozoans and examined by confocal microscopy. Multiple serial sections were captured allowing three-dimensional computer reconstructions to be developed. The spores were found to consist of eight structural valve cells, four capsular cells including spherical polar capsules, and two germinative sporoplasms, each comprising a primary and secondary cell.

Introduction

Proliferative kidney disease (PKD) is well recognised as being a highly significant parasitic disease of salmonid fish, posing a high financial burden on the aquaculture sector.¹ Primarily affecting first season freshwater fish, PKD has become endemic in areas of Western Europe and North America. Studies revealed that freshwater invertebrates of the phylum Bryozoa - known colloquially as "moss animals" - acted as alternate hosts, and the organism was eventually named *Tetracapsuloides bryosalmonae* and placed in the new class Malacosporea within the phylum Myxozoa.²

Materials and methods

Colonies of the phylactolaemate bryozoan, *Fredericella sultana* were collected from the inlet to a fish farm in Southern England known to be endemic for PKD. The specimens were laboratory reared at 18°C on an artificial diet comprising protozoa and algae.³ Following three weeks of observation using dissecting and inverted microscopes, characteristic signs of malacosporean parasitic infection were noted in the bryozoans. Rainbow trout (*Oncorhynchus mykiss*) were experimentally exposed to material released from the bryozoan colonies and developed clinicopathological signs of PKD.

Samples of *F. sultana* containing conspicuous spores of *T. bryosalmonae* were carefully teased apart on a slide under a dissecting microscope. The first sample examined was stained with a polysaccharide specific fluorescent dye, subsequent samples being stained with either a non-polar lipophilic stain or a nucleic acid dye. Following staining, the microscope slides were coverslipped and sealed with nail varnish. The slides were then examined using the optical sectioning function of a Leica confocal laser scanning microscope. Images captured during the examinations were processed using Leica Confocal Software to generate three-dimensional (3D) representations of the spore structure of *T. bryosalmonae*.

Results

Eighteen approximately spherical *T. bryosalmonae* spores of mean diameter 19.16 µm (s.d. = 1.15) were identified and analysed using the range of fluorescent dyes. All specimens were seen to possess four characteristic spherical polar capsules arranged within four capsular cells forming a cruciate pattern at one aspect of the spore (Fig. 1). Several spores were observed to have undergone extrusion of between one and four polar filaments resulting in morphological changes to the architecture of the spore (Fig. 2).

Surrounding the capsular cells were four well-defined valve cells, while an additional four valve cells at the other aspect of spore fulfilled the complement of eight. The junctions between adjacent cells could be readily visualised in the samples stained with the polysaccharide specific dye (Figs. 1 & 2) while the nucleic acid stain (Fig. 3) confirmed the presence of 12 nuclei in the outer layer of the structure (eight valve cells and four capsular cells). The lipophilic dye sufficiently penetrated the structure to allow examination of the two germinative sporoplasms of the spores (Fig. 4) - each possessing the nucleus of a primary and secondary cell - while also demonstrating multiple lipid vesicles of diameter 500 nm throughout the spores.

Discussion

Due to the minimal processing of material required during these procedures, few artefacts could develop to alter the morphology of the spores before examination.⁴ Thus, structures alluded to in previous electron microscopical studies could now be viewed unadulterated *in situ*.^{5,6} Three-dimensional reconstructions of the image slices obtained using this technique have allowed computer modelling to propose a realistic conformational architecture of the spore of *T. bryosalmonae* (Fig. 5). Future examination of other malacosporean spores - such as *Buddenbrockia plumatellae* and fish stages of *T. bryosalmonae* - utilising similar techniques will provide a powerful method to compare species of the class Malacosporea.

References

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Fig. 1: 3D confocal representation of a *T. bryosalmonae* spore from which a polar filament has been extruded from one of the polar capsules.

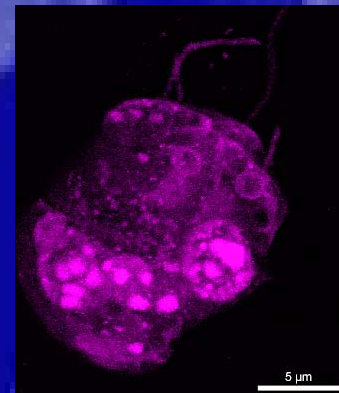


Fig. 2: Four polar filaments have been extruded from the spherical polar capsules. Sporoplasmic constituents could also be visualised in this 3D reconstruction.

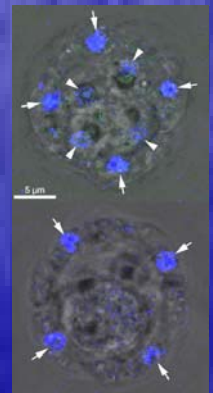


Fig. 3: Two sections with nuclei stained; four capsular cell nuclei (arrowheads) and eight valve cell nuclei (arrows) could be seen.

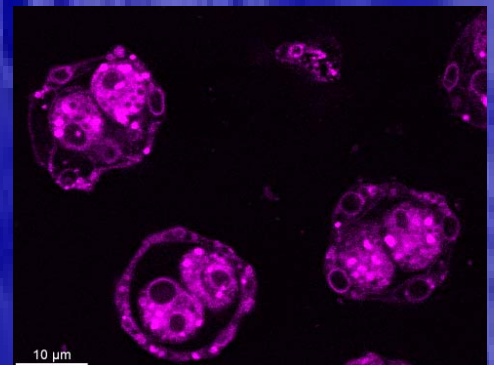


Fig. 4: Section of three spores demonstrating internal sporoplasms.

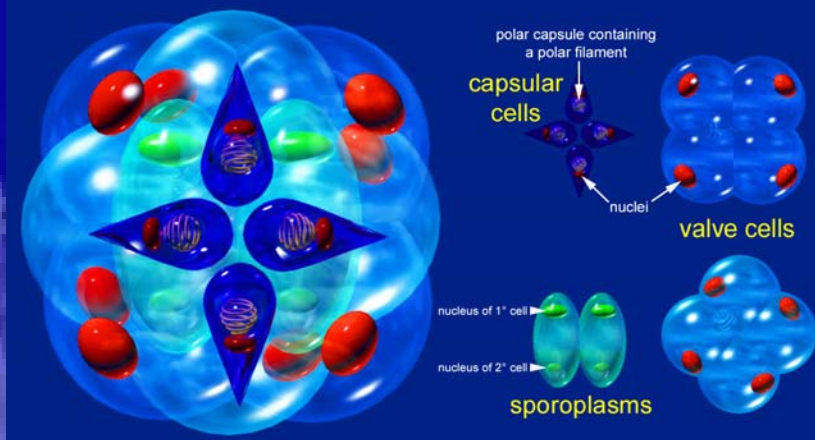


Fig. 5: Three-dimensional computer generated models of *T. bryosalmonae* spore structure.

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