Vol. 38: 219–224, 1999 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published November 30

Experimental detection of the actinospores of *Myxobolus pseudodispar* (Myxosporea: Myxobolidae) in oligochaete alternate hosts

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ABSTRACT: The development of *Myxobolus pseudodispar* Gorbunova, 1936, an intracellular myxosporean muscle parasite of the roach *Rutilus rutilus* L., was studied in experimentally infected oligochaetes. In one experiment, uninfected *Tubifex tubifex* Müller and *Limnodrilus hoffmeisteri* (Claparéde) were exposed to mature spores of *M. pseudodispar*. Triactinomyxon spores developed both in *T. tubifex* and *L. hoffmeisteri* specimens. Triactinospores were first released from the oligochaetes 76 d after initial exposure. At that time, pansporocysts containing 8 triactinospores were located in the gut epithelium of experimentally infected oligochaetes, but free actinosporean stages were also found in their gut lumen. Each triactinospore had 3 pyriform polar capsules and an elongated cylindrical sporoplasm with 8 secondary cells. The spore body joined the 3 caudal projections with a relatively long style. One of the 3 caudal projections was shorter than the other two. The total length of the triactinospore was on average 206.5 µm.

KEY WORDS: *Myxobolus pseudodispar* · Myxosporea · *Rutilus rutilus* · Development · Triactinomyxon stage · *Tubifex tubifex* · *Limnodrilus hoffmeisteri*

INTRODUCTION

Myxobolus pseudodispar, a species described by Gorbunova (1936), is a myxosporean commonly occurring in the skeletal muscles of the roach *Rutilus rutilus* L. in Hungarian lakes, rivers and ponds (Baska 1986). Some aspects of its location and intrapiscine development were also studied by Baska (1986). Nothing was known, however, about its extrapiscine developmental stage.

The extrapiscine phase of a myxosporean was first studied by Wolf & Markiw (1984), who revealed that the extrapiscine development of *Myxobolus cerebralis* Hofer took place in an oligochaete alternate host (*Tubifex tubifex* Müller). Following that pioneering work, several other papers were published to support its finding. These papers (El-Matbouli & Hoffmann 1989, 1993, Ruidisch et al. 1991, Styer et al. 1991, El-Matbouli et al. 1992, Grossheider & Körting 1992, Benajiba & Marques 1993, Kent et al. 1993, Yokoyama et al. 1993, 1995, Uspenskaya 1995, Trouillier et al. 1996, Yokoyama 1997) revealed that in each case various *Oligochaeta* spp. or *Polychaeta* sp. (Bartholomew et al. 1997) acted as alternate hosts in the development of various myxosporean species.

Recently the life cycles of 7 species from the genera *Myxobolus*, *Thelohanellus* and *Sphaerospora* have also been experimentally studied in our laboratory (El-Mansy & Molnár 1997a,b, El-Mansy et al. 1998a, Székely et al. 1998, Molnár et al. 1999a,b).

The work presented in this paper is part of continuing experimental life cycle studies conducted on the most common myxosporeans of Hungarian fishes. In the experiments reported here, the development of *Myxobolus pseudodispar* was followed in *Tubifex tubifex* and *Limnodrilus hoffmeisteri* alternate hosts.

MATERIALS AND METHODS

Myxospores of *Myxobolus pseudodispar* were collected from mature muscle cysts of 2 to 5 yr old roach *Rutilus rutilus* specimens seined in Lake Balaton, Hun-

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gary. Pieces of the muscle were cut, compressed between 2 glass plates, and examined for the presence of myxosporean plasmodia. Plasmodia located intracellularly within muscle cells were separated and opened with a needle under a stereomicroscope. Mature spores obtained from plasmodia were identified and counted. A total of 190 000 spores of *M. pseudodispar* were collected.

Oligochaetes *Tubifex tubifex* Müller and *Limnodrilus hoffmeisteri* (Claparéde), identified according to Brinkhurst (1963), were collected from the outlet of a duck farm in Szarvas, Hungary, where no fishes live. This parasite-free oligochaete stock containing members of the 2 species in about the same number was transferred to sterilised mud, and propagated in the laboratory in aerated aquaria. The worms were fed on some drops of granulated fish food.

The experiment started on 4 September 1998, when 100 specimens each of *Tubifex tubifex* and *Limnodrilus hoffmeisteri* were placed into a small plastic cup of 500 ml volume, to which spores of *Myxobolus pseudodispar* were added. The calculated spore count per oligochaete was a total of 1000. The temperature of the room during the experiment varied between 20 and 23°C. All dishes were permanently aerated and regularly supplied with fresh water to prevent evaporation and to refresh the water for the oligochaetes. Starting from 42 d post-exposure (p.e.), water from the dishes was filtered weekly through a fine mesh of 21 µm pore size. The filtrates were taken up in a drop of water and examined for the presence of actinospores. On Day 76 p.e. 150 oligochaetes were placed into 2 ml cell-well plates as described by Yokoyama et al. (1991), and after 1 d of incubation they were examined for the release of actinospores under a stereomicroscope. Triactinospores released by the oligochaetes were examined under a coverslip. They were recorded on videotapes and digitized pictures were taken with the help of the IMAGO® program as described by Székely (1997). Photographs were taken, drawings made and measurements of 47 actinospores recorded. In the description, all measurements are given in µm. The actinosporean stage of *M. pseudodispar* was described using the terminology of Janiszewska (1957) as modified by Lom et al. (1997). After the first release of actinospores live *T. tubifex* and *L. hoffmeisteri* specimens were also examined for actinosporean stages under a compound microscope. After finding the first released triactinospores 2 infected *T. tubifex* and 1 *L. hoffmeisteri* specimen were fixed in 5% glutaraldehyde for 1.5 h and post-fixed in 2% osmium tetroxide for 2 h, washed several times with cacodylate buffer, dehydrated and embedded in Durcupan ACM resin. Semithin sections (0.5 to 1 µm) were made and stained with 0.1% toluidine blue solution.

RESULTS

Light microscopy

Actinospores of *Myxobolus pseudodispar* were released from both *Tubifex tubifex* and *Limnodrilus hoffmeisteri*, but no infection was found in the control tanks of the same oligochaete species. Of 150 specimens examined in cell-well plates, 4 *T. tubifex* (5.3%)

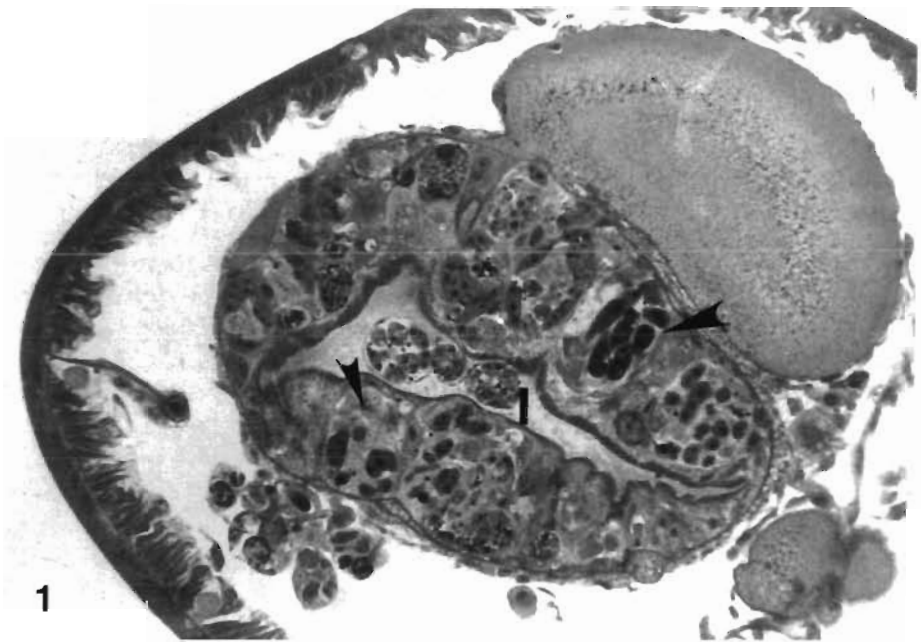

Fig. 1. Cross-section of *Tubifex tubifex* infected by *Myxobolus pseudodispar*. The intestine of the oligochaete is heavily infected with pansporocysts (arrowheads) located in the epithelium. Some released pansporocysts are found also in the intestinal lumen (l). Semithin section. ×250

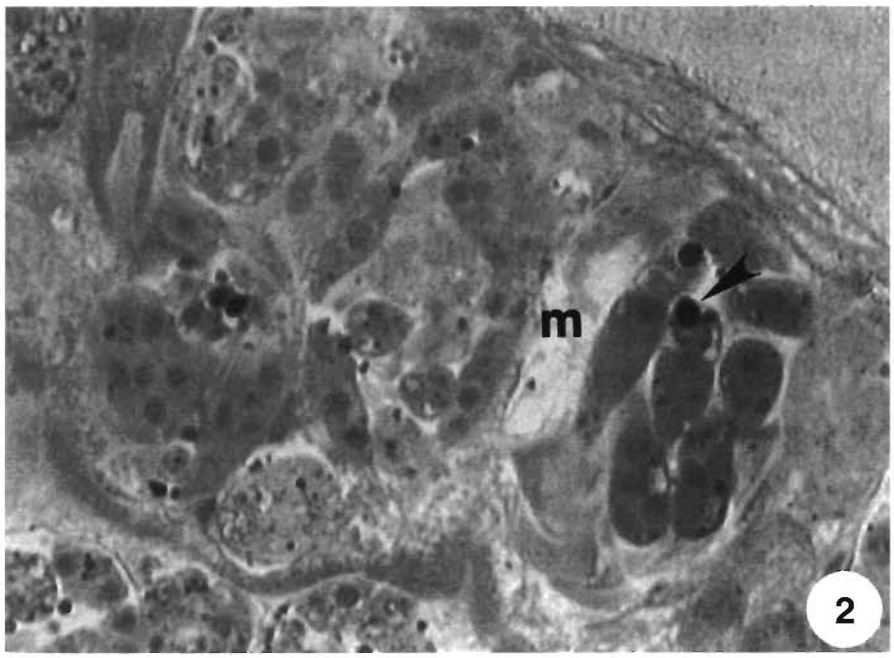

Fig. 2. Cross-section of *Tubifex tubifex* infected by *Myxobolus pseudodispar*. Enlarged part of Fig. 1 A spansporocyst containing 8 actinospores is located in the gut epithelium. Inside the pansporocyst sporoplasm, polar capsules (arrowhead) and folded membranes of the caudal processes (m) of triactinospores are seen. Semithin section. ×2000

and 2 *L. hoffmeisteri* (2.7%) proved to be infected. During the experiment, only triactinomyxon-type actinospores were obtained from all infected worms. The release of triactinospores lasted for about 30 d. In infected live oligochaete specimens placed under a coverslip, pansporocysts in the gut epithelium and free spores in the gut lumen of the worms were easily seen under a compound microscope. No infection was found in the control oligochaete stock examined in the same numbers.

Histology

Pansporocysts of *Myxobolus pseudodispar* were mostly found in the gut epithelium, but pansporocysts and free spores released into the intestinal lumen through the damaged gut epithelium were also seen in semi-thin sections (Fig. 1). Eight triactinospores could be counted in a pansporocyst. In horizontally sectioned triactinospores the spore body with the sporoplasm and the dark-stained polar capsules could also be recognised (Fig. 2) Inside the sporoplasm large, round secondary cells were distinguishable. The free part of the spore body inside the pansporocyst was filled with the folded styles and caudal processes of the actinospores, which could easily be differentiated from the spore body by their pale colour.

Description of triactinospores. Triactinospores (Table 1, Figs. 3 & 4) released from the tubificid body and floating in the water were characterised by 3 pyriform polar capsules, a sporoplasm, a style and 3 caudal processes (Fig. 4). Polar capsules, pyriform in shape, were 5.1 (4–6) µm in length and 3 µm in width. The cylindrical sporoplasm, 45 (42–48) µm long and 15.8 (12–18) µm wide, contained 8 spherical secondary cells 7.6 (6.4–8) µm in diameter (Fig. 3 inset). The style was relatively long, 157.3 (145–173) µm in length. Its width measured 15.8 (12.8–18) µm at the middle. Caudal processes were unequal in length, one of them being shorter. They were slightly bent, tapering toward the end and terminating in a sharply pointed end. The length of the 2 longer caudal processes was 196 (190–204) µm, that of the shorter one 127.2 (104–144) µm. Their width at the middle was 13.6 (12–16) µm.

Table 1. *Myxobolus pseudodispar*. Measurements of the triactinospores (on the basis of 47 triactinospores) in µm

Measure		Mean ± SD	Minimum	Maximum
Total length		206.5 ± 15.6	179.2	244
Polar capsules	Length	5.1 ± 0.5	4	6
	Width	3.0 ± 0.0	3	3
Sporoplasm	Length	45.0 ± 2.2	42	48
	Width	15.8 ± 1.1	12.8	18
Spore body	Length	50.4 ± 2.4	47	53
	Width	15.8 ± 1.1	12.8	18
Style	Length	157.3 ± 14.8	145	173
	Width	15.8 ± 1.1	12.8	18
Caudal processes				
Longer	Length	196.6 ± 5.7	190	204
	Width	13.6 ± 1.7	12	16
Shorter	Length	127.2 ± 14.8	104	144

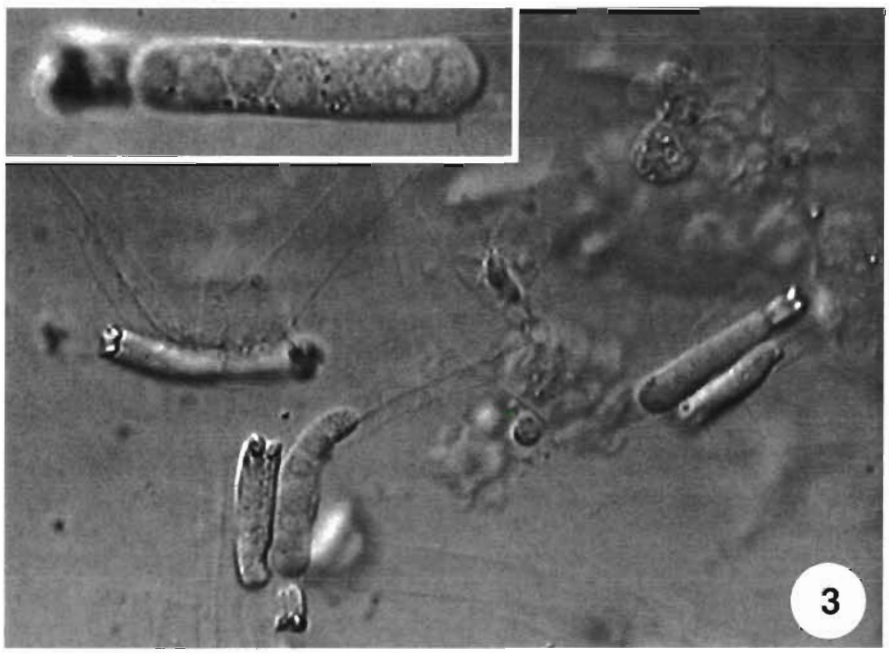

Fig. 3. *Myxobolus pseudodispar*. Triactinospores floating in the water. ×500. Inset: Spore body of the triactinospore with sporoplasm containing 8 sporoplasm cells and with polar capsules at the apical end. ×1200

The length from the apical point of polar capsules to the end of sporoplasm (spore body) measured 50.4 (47–53) µm. The total length from the polar capsules to the end of style (spore body with style) measured 206.5 (179–244) µm.

Differential diagnosis

Both the myxospores and the actinospores of *Myxobolus pseudodispar* can readily be differentiated from those of other species by their asymmetric forms. The triactinomyxon stages of this species resemble other known triactinomyxospores of myxosporean species. However, they are distinguishable by the low number of secondary cells in the sporoplasm and by the shorter size of 1 of the caudal processes. The sporoplasm of this species has only 8 large sporoplasm cells and the third caudal process measures only two-thirds of the 2 others.

The triactinomyxons obtained in this experiment are probably identical to one of the triactinomyxon types (T2) detected by El-Mansy et al. (1998b) during a survey of actinospore infection of oligochaetes in a fish farm near Budapest.

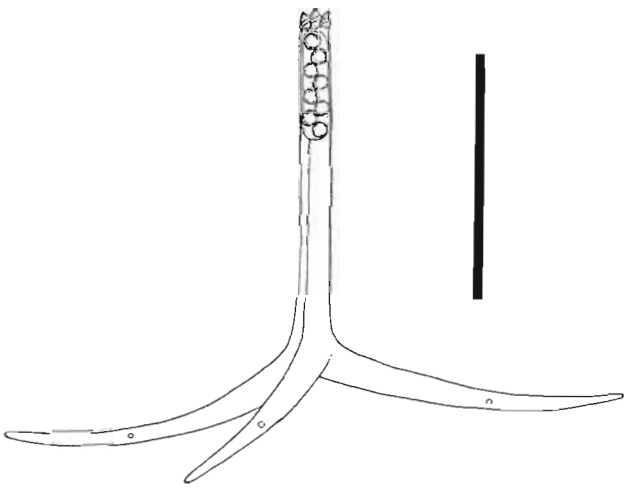

Fig. 4. Schematic drawing of the triactinospore of *Myxobolus pseudodispar*. Bar = 100 µm

DISCUSSION

The species *Myxobolus pseudodispar* is a rather common parasite of the roach in Hungary (Molnár 1979, Baska 1986, Székely & Molnár 1996–1997). The validity of the species was questioned by Lom & Dyková (1992), who regarded it as a synonym of *M. cyprini*. In the typical fish hosts (*Cyprinus carpio* and *Rutilus rutilus*) clear differences exist in the spore morphology (Molnár & Kovács-Gayer 1985, Baska 1986), which supports the opinion represented by Gorbunova (1936) and Donec & Shulman (1984); therefore, we regard *M. pseudodispar* as a valid species. In spite of its common occurrence, little is known about its pathogenicity. No studies have been done on the intraoligochaete development of this species. Data obtained in this study show that the intraoligochaete development of this parasite follows the same pattern as described by Wolf & Markiw (1984) for *M. cerebralis*. In the present experiments the development of *M.*

pseudodispar was successfully completed in both *Tubifex tubifex* and *Limnodrilus hoffmeisteri*, in which triactinomyxon-type spores developed. In their shape and size the spores resembled most of the triactinospores known from experimental studies on *Myxobolus* species (*M. cerebralis*, *M. cotti*, *M. carassii*, *M. drjagini*, *M. portucalensis*, *M. hungaricus*), but due to the low number of secondary cells in the sporoplasm and by the shorter length of one of the caudal processes they distinctly differed from the known triactinospores.

According to El-Matbouli et al. (1992), the intraoligochaete development of *Myxobolus cerebralis*, *M. cotti* and *M. carassii* took 80 to 120 d, while for *M. drjagini*, *M. hungaricus* and *M. portucalensis* El-Mansy & Molnár (1997a,b) and El-Mansy et al. (1998a) reported development times of 91, 102 and 160 d, respectively. The patent period of *M. portucalensis* was somewhat shorter than that of the above-listed species.

The prevalence of infection (5.3% in *Tubifex tubifex* and 2.7% in *Limnodrilus hoffmeisteri*) proved to be relatively low compared to the high levels observed in previous experiments on *Myxobolus drjagini*, *M. portucalensis*, *M. hungaricus* and *M. dispar* (El-Mansy & Molnár 1997a,b, El-Mansy et al. 1998a, Molnár et al. 1999a) in our laboratory. From a single experiment it is impossible to draw conclusions as to whether the alternate hosts, the myxospores or some other factors are responsible for this low prevalence.

Histological studies prove that *Myxobolus pseudodispar* develops in the gut epithelium of the oligochaete alternate host, from where its pansporocysts and spores are released into the lumen and subsequently excreted with the faeces.

Up to now, the intraoligochaete life cycle of about 20 species has been elucidated. However, a technique enabling consistently successful laboratory infection is known only in the case of *Myxobolus cerebralis*. The species *M. pseudodispar* is not ideal for use in experiments, as its relatively small, intracellular plasmodia can be collected and identified only with great difficulty. Reinfection of the fish host with actinospores is also difficult to achieve, as roaches reared infection-free are not yet available. At the same time, the collected myxospores and the obtained actinospores provide excellent material for expanding the molecular biological studies that have already been done on other species (Andree et al. 1999).

Acknowledgements. The study was supported by the Hungarian Scientific Research Fund (OTKA, projects No. T 020044 and T 029200). The authors thank Ms Zsuzsa Kis for her help in collecting and culturing tubificids, and are grateful to the Balaton Fisheries Co. for providing fish.

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Editorial responsibility: Wolfgang Körting, Hannover, Germany

Submitted: May 17, 1999; Accepted: September 3, 1999
Proofs received from author(s): November 16, 1999