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LIGHT, ULTRASTGRUCTURE AND CYTOCHEMICAL STUDIES ON *MYXOBOLUS* SP. (MYXOZOA: MYXOBOLIDAE) INFECTING THE GILL TISSUE OF THE NILE FISH *LABEO NILOTICUS* AT ASSIUT LOCALITY EGYPT.

(With One Table and 12 Figures)

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دراسة التركيب الدقيق والضوئى والسيتوكيماء لطفيلي ميكزوبولس الذى يصيب السمكة النيلية ليبونيلوتيكس باقليم اسيوط - مصر

عبدالله الحوت

اوضحت الدراسة وجود طفيلى ميكزبولس فى خياشم السمكة النيلية ليبونيلوتيكس باقليم اسيوط- وقد وصفت حويصلات وجراثيم هذا النوع وصفا تفصيلا وقورونت بالانواع الاخرى القريبة لها من نفس الجنس واضحت دراسة السيتوكيماء وجود كميات من الجليكوجين والبروتين فى الاسبوروبلاست وهذا يفسر مدى احتياج هذه الخلايا للطاقة التى تساعد على تكوين الجراثيم كما ان الفراغ اليودى فى اسبوروبلازم الجرثومة يحتوى على الجليكوجين كما انه دائرى الشكل. وقد اوضحت الدراسة باستخدام الميكروسكوب الاكترونى الماسح السطح الاملس لجدار البوغية وجود الكبسوليتن القطبيتين متساويتين فى الحجم واماميتين وقد بينت الدراسة باستخدام الميكروسكوب الاكترونى النافذ ان تكوين الكبسوليتن القطبيتين متزامن وقبل نضج الاسبوروبلازم وان الخلايا الجدارية تتميز بوجودها فى الناحية الطرفية والاسبوروبلازم يتميز بتجمع مواد الجليكوجين والحبيبات الكثيفة الكترونيا

SUMMARY

Myxobolus sp. (Myxozoa: Myxosporea) was recovered from gill tissues of the Nile fish *Labeo niloticus* at Assiut locality, Egypt. Mature spores were elliptical in shape, with two equal polar capsules situated anteriorly and occupied nearly more than half length of the spores. Each spore measured 10.4-14.0 μm in length and 8.0-11.3 μm in width. Polar capsules were 5.5 – 7.3 μm in length and 3.5-4.5 μm in width. They

occupied nearly more than half length of spores. Semi-thin-sections revealed that the plasmodium was surrounded by an outer wall of the host origin and inner wall of the parasite and contained numerous spores at different stages of development. Cytochemical studies showed that the spores and sporoblasts contained large amount of glycogen and protein. Best's carmine stain showed the presence of iodinophilous vacuoles in the sporoplasm of spores as a rounded structure. Scanning electron microscope revealed the smooth nature of the spore valve surface. The polar capsules, shell valves and sutural ridge line are represented by anterior and posterior grooves. The transmission electron microscope revealed that the mature spores have two valves arising from the valvogenic cells and joined by the sutural line.

Key words: *Myxosporea, Myxobolus* sp. *and Labeo niloticus*

INTRODUCTION

Myxosporea are mainly parasites of fish. The myxosporean *Myxobolus* sp. is a common parasite infecting a wide variety of organs and tissues in many families of fishes. Infection may cause serious disease and high mortality among fishes (Molnar, 1982; Lom, 1984, Cone & Wiles, 1985, Stehr & Whitaker, 1986 and El-Matbouli *et al.*, 1992). Today *Myxobolus* sp. have special attention than before, since they may infect man through consumption of under cooked infected fishes (Boreham *et al.*, 1998). *Myxobolus* sp. have been decribed from fishes by many authors such as (Walliker, 1969; Fahmy *et al.*, 1971, 1975; Kent & Hoffmann, 1984; Abed, 1987 and Ali *et al.*, 2002).

The ultrastructure of *Myxobolus* sp. has been studied by many authors such as (Lom & de Puytorac, 1965; Current, 1979; Mitchell *et al.*, 1985; Pulsford & Mathews, 1982; El-Matbouli *et al.*, 1990; Kent *et al.*, 1993 and Abed, 1999; 2005). However, for the best of the author,s knowledge, no further details on this species have so far been reported.

The present work was desined to study the light microscopy, ultrastructure and cytochemistry of *Myxobolus* sp. infecting the gill tissues of the Nile fish *Labeo niloticus* at Assiut locality

MATERIALS and METHODS

Forty adult fish *Labeo niloticus* were collected during summer, 2006 from the main River Nile at Assiut, Upper Egypt. The collected fish ranged from 30-50 cm. in length and were examined for myxosporean parasites. The irregular shape of polysporic and histozoic plasmodia were observed macroscopically. They were found in gill tissues of fishes and processed as follows:

For light microscopy: Wet mounts were prepared and examined, others were dried and stained with Giemsa stain. Lugol's iodine solution was added to the wet mounts to observe the presence of iodinophilous vacuoles in the sporoplasm. The polar filaments were readily extruded in wet mounts by addition of 5% KOH. and photographed for measurement and description purposes. Other plasmodia were fixed in formol alcohol, then embedded in paraffin wax as usual. Paraffin sections were stained with certain histochemical reagents (periodic acid Schiff's reagents (P.A.S.), P.A.S. reactions with diastase control, Bests carmine, and Bromophenol blue stains).

For scanning electron microscopy (SEM); pieces of the parasitized fish containing plasmodia, were fixed in a solution of 5% glutaraldehyde at pH 7.4 for 3-4 hr., washed in Na-phosphate buffer (pH 7.4), following maceration using needles to release spores and allowed to settle into coated cover slips. Dehydration of samples were carried out by ascending grades of ethanol series then processed in a critical point drying apparatus. The cover slips were subsequently mounted on copper studs, gold coated, and examined using scanning electron microscope (J. EOL JSM-5400LV).

For transmission electron microscopy (TEM); plasmodia were fixed in phosphate-buffered 5% glutaraldehyde (pH 7.4) for 3-4 hr., post-fixed in 1% (osmium tetroxide) for 2 hr., washed in the same buffer, dehydrated in ascending grades of ethanol series and finally embedded in Epon. Ultra-thin sections were contrasted with urinyl acetate and lead citrate to be examined with a transmission electron microscope (Jeol CXII) at 80 Kv.

RESULTS

Out of the surveyed forty fishes *Labeo niloticus* eight (20%) showed parasitic infection of gill tissue. Varied sized yellowish white cystic structure, elongated to oval in shape, were found embedded in the

gill tissues. Cysts were attached firmly to the primary gill lamellae. The larger parasitic cysts measured (1.3-6 x 0.4 –0.8 mm), while the smaller ones measured (0.4 – 1.0 x 0.3 – 0.5 mm) and were found attached to the secondary gill lamellae. The branchial arch was free from parasitic infection. The infected gill tissues were pale.

Wet mounts (imprints) prepared from the infected gill tissues revealed the presence of numerous elliptical shaped spores. They were blunt with slightly narrow anterior end and slightly broad posterior one, ovoid shaped in front view and spindle-shaped in sutural view and lateral view (Fig. 1).They measured 10.4-14 μm x 8.0 – 11.3 μm. Two equal oval to pyriform polar capsules converging anteriorly (Fig. 2), each measured, 5.5 - 7.3 x 3.5 – 4.5 μm. The ratio of polar capsules to spore length equals 0.52. The polar filament measured 25-40 μm in length when it is fully extruded, but when it is resting inside the polar capsule, it consists of 5-6 coils.

When Lugol's iodine was added, a brownish rounded to elliptical mass was detected within the sporoplasm of the spore, commonly known as iodinophilous vacuole, measuring 2.9-3.2 μm and corresponds to a vacuole observed in stained specimen. The sporoplasm is finely granulated with two nuclei (Fig. 2) each measuring, 1.1-1.9 μm.

Histological findings:

Varied sized parasitic cysts were recognized, attached to the primary gill lamellae of all infected fishes. The cyst wall was partially built-up of the host tissue and separated by a narrow space from the subjacent plasmodium membrane (Fig. 3). Within the plasmodia vegetative forms, developing sporonts, sporoblasts, maturing and mature spores were found. The mature parasitic spores tended to locate toward the plasmodial center (Fig. 4).

Periodic acid Schiff's (P.A.S.) stained sections showed that the cyst wall, was P.A.S. positive. The sporoblasts and iodinophilous vacuole within the sporoplasm of spores were deep red in colour P.A.S. positive (Fig. 5). Some sections were exposed to diastase to be digested for 30-60 minutes at 37ºC followed by P.A.S. stain, the result was that the outer layer of the cyst wall became negative, except the mucous peripheral cells which remain positive. The sporoblasts and iodinophile vacuole changed their red colour as a result of digestion of the glycogen present in these structure (Fig. 6).

Best's carmine stained sections revealed that the sporoblasts and the iodinophilous vacuole of spores stained deep red in colour (Fig. 7).

Bromophenal blue stained sections showed that the sporoblasts and spores stained blue in colour (Fig. 8).

Scanning electron microscopy:

Frontal view of the scanned spores of this species revealed the smooth nature of both spore valves and inconspicuous suture separating them. The two anteriorly situated polar capsules were distinct (Fig. 9). The valve had parallel striations. Some variations in the pattern were evident, each striation did not extend over the entire length of the spore and was observed to be cross-linked or branched (Figs. 9, 10). The anterior polar capsules, shell valves and sutural ridge, represented by anterior and posterior grooves were also evident

Transmission electron microscopy:

Ultra-thin sections prepared from the parasitic cysts revealed obviously the attachement of the plasmodial membrane to the cyst wall. The host cells contributed apparently in the formation of the cyst wall. Sporoblasts, developing sporonts were located at the periphery of plasmodium, while maturing spores were located toward the plasmodium center (Figs. 3, 4).

Electron micrographs showing the spore developmental stages (stages of sporogenesis) gave an evidence that the development of two spore polar capsules was synchronized and consisted of an electron-dense outer zone, two adjacent translucent zones and cortex containing polar filaments. Polar filaments in the early developing polar capsules (primordial filaments) were loosely coiled. The nearly mature spores revealed the presence of two distinct polar capsules (with more synchronization of development) containing regularly peripherally arranged filament coils (Fig. 11). The well-developed mature polar capsules possessed enlarged 5-6 polar filaments coiled in a dense granular matrix and arranged perpendicular to the length of the mature capsule. The mature spores have two valves arising from the valvogenic cells and joined by the sutural line (Fig. 12). The valvogenic cells revealed an accumulation of electron-dense bodies “valve-forming material” especially in regions of sutural ridge. The sporoplasmic cells were ensheathed by valvogenic cells and consisted of pairs of cells. By time they became fashioned together. The cytoplasm contained light and dark electron dense-bodies, the sporoplasm filled all the space beneath polar capsules and partially extended between them.

Table 1: Comparison between the present parasite and other related species of myxosporean, *Myxobolus* spp.

Characters	*Myxobolus nilicus* Fathy *et al.* (1971)	*M. sp.* Type (2) Fahmy *et al.* (1975)	*M. naffari* Abdel Ghaffar, 1998, Ali *et al.* (2002)	*M. labi* Abed (2005)	The present material
Host	*Labeo niloticus*	*L. niloticus*	*L. niloticus & B. Bynni*	*L. nilotics*	*L. niloticus*
Habitat	Parasitic cyst from fin rays	Gill filaments	Mouth B. bynni & gill of *L. nioticus*	Primary gill Filaments	Gill filaments
Locality	River Nile at Assiut	River Nile at Assiut	R. Nile at Beni-Suef	R. Nile at Assiut	R. Nile at Assiut
Spores					
Shape	Oval	Oval	Subspherical to elliptical	Oval	Subspherical to elliptical
Size:	10.25-11.75x 6/3-7.8 µm	10.15-13.1 x 7.8-10.6 µm	10.4-12 x 7.2-9.6 µm	9.2-11.5x5.2-7.5 µm (10.4-6.4 µm)	10.4 – 14.0 x8.0-11.3 µm)
Polar capsules					
Shape	2 unequal	2 large & equal	2 large & equal occupied half length of spores	2 equal & pyriform to oval	2 equal pyriform Occupied more than half length of spores
Size:		5.2-6.98 x 3.14-3.64	4.6-6.4 x 2.4-4 µm	3.1-4 x 1.5-2.4 µm (3.6x2 µm)	5.5- 7.3 x 3.5 – 4.5 µm
Long	5.2-6.8 x 2.5-3.3	-	-	-	
Short	2.6-4.3 x1.4-1.7 µm				
Polar filament					
Length		5.6-6.3 µm	-	2.3-3.5 µm	25 – 40 µm
Long	76-84 µm				
Short	23-26 µm				
No. of coils			6-7 coils	4-5 coils	5 – 6 coils
Iodinophilous vacuole	Round mass	Present	-	Elliptical measured *1.3-1.9 µm (1.5 µm)*	Elliptical measured, 2.9 – 3.2 µm
Sporoplasm	-	-	-	Finely granulated	Finely granulated
Nuclei	-	-	-	*2* nuclei each. *measured 1.1.4 µm* (1.2 µm)	Two each measured, 1.1 – 1.9 µm
Structure of the cyst	-	-	-	Outer from host & inner plasmodia into 2 layer, an outer minute process & an inner metrocyte	Outer from host & inner plasmodia contaning germinal layers & maturing spores, tend to be located inward

FIGURE LEGNES

Fig. 1: Photomicrograph showing fresh smears of *Myxobolus* sp. The spore is elliptical in shape in ventral view (arrow) and spindle-shaped in lateral view (asterisk *). X 1000.

Fig. 2: Photomicrograph showing spores of *Myxobolus* sp, They are elliptical in shape with two equal polar capsules located anteriorly (arrow heads). The sporoblasm is finely granulated with two smaller nuclei (arrow) and contains elliptical shaped iodinophilous vacuoles (asterisk *). X 1000.

Fig. 3: Photomicrograph showing a large pseudocyst enclosing a plasmodium (P) which contains numerous spores at different stages of development. The plasmodium ectoplasm is impacted with germinal layers (asterisk). The developing and maturing spores tend to locate toward the plasmodium center Toluidine blue stain. X 200.

Fig. 4: Photomicrograph showing higher magnification of the pseudocyst center. The numerous spores (arrow), have spindle-shaped in lateral view, and ovoid shaped in ventral view Toluidine blue stain. X 1000.

Fig. 5: Photomicrograph showing T.S. of the cyst. Parasitic germinal layer (nucleated layer containing sporoblasts) stained red in colour and the iodinophilous vacuoles in sporoplasm of spores stained deep red (arrow head). P.A.S. stain X 400.

Fig. 6: Photomicrograph showing the iodinophilous vacuole (*) of *Myxobolus* sp. after digestion with diastase. X 400.

Fig. 7: Photomicrograph showing the rounded structure of iodinophilous vacuole (*) of *Myxobolus* sp. stained with Best's carmine stain. X 400.

Fig. 8: Photomicrograph showing spores of *Myxobolus* sp. stained with Bromophenol blue stain. X 400.

Fig. 9: Scanning electron micrograph showing spore of *Myxobolus* sp. with two anteriorly polar capsules (*), smooth shell valves (arrow). X. 7500

Fig. 10: Frontal view of a spore showing inconspicuous sutural ridge and smooth valves. The valve striation do not extend over the entire length of spore (*) scanning electron micrograph. X 7500.

Fig. 11: Developing spores of *M.* sp, the spore shows synchronous development of polar capsules. The polar capsule exhibits two electron layers. The polar filaments (*) within the polar capsules are loosely coiled. The valve forming material (vm) in valvogenic cells. Transmission electron micrograph. X 6700.

Fig. 12: A mature spore showing two valves (arrows) arised from the two valvogenic cells and joined by a sutural line. Note the accumulation of valve-forming material (*) in the cytoplasm of valvogenic cell. Transmission electron micrograph. X 8000.

DISCUSSION

Since the spores of the present studied parasite possess two anteriorly situated polar capsules and the sporoplasm contains an iodinophilous vacuole, the parasites belong to family Myxobolidae Thelohan, 1892 and genus *Myxobolus* Bütschli, 1882.

The macroscopic observation of the parasitic cysts were similar to those described in previous studies on myxosporean parasites (Fahmy *et al.*, 1971, 1975 and Abed, 2005).

Gill parasitic myxosporeans, including *Myxobolus* sp. were commonly described in gill filaments of infected fishes (Waliker, 1969; Lom & Molnar, 1983, Fahmy *et al.*, 1971, 1975; Kent & Hoffmann, 1984; Rocha *et al.*, 1992; Azevedo & Matos, 1996; Ali *et al.*, 2002 and Abed 2005).

When the present species is compared in details with the previously described species such as *Myxobolus niloticus* described by Fahmy *et al.* (1971), *Myxobolus* sp. type (2) described by Fahmy *et al.* (1975), *Myxobolus naffari* described by Ali *et al.* (2002) and *Myxobolus labi* described by Abed (2005), it was found to be more or less similar to *Myxobolus naffari* described by Ali *et al.* (2002), although the above mentioned study had not presented a detailed microscopic study of that species Table (1). The present study is the first to describe in detail a *Myxobolus naffari* species infecting the gills of the Nile fish *Labeo niloticus*.

The presence of large amount of carbohydrates and protein in the germinal layer (nucleated layer containing sporoblasts) may explain the function of these cells, since they are highly specialized, thus they need more energy to produce offsprings in the form of spores.

In the present study, the presence of a red rounded structure in the sporoplasm of spores as shown by Best's carmine stain, may indicate that this structure is a store house for glycogen which may be used as a source of energy for establishment and further development of the parasite.

The plasmodial wall was delimited by a membrane similar to *Myxobolus* sp. described by Desser & Paterson (1978).

In the present study the elliptical shape of the spores, smooth valve surface, slit like opening and suture lines were similar to those described by Desser & Paterson (1978), Mitchell *et al.* (1985), El-Mathouli *et al.* (1990) and Dzulinsky *et al.* (1994).

The sequence of events responsible for development of polar capsules in the present study were identical to those reported for several species of Myxosporea (Lom & de Puytorac, 1965; Current *et al.*, 1979 and Abed 2005).

The gradual reduction of valvogenic cells till they were completed in mature spores and the formation of sutures joined the valves was similar to that reported in other myxosporeans sp. (Desser & Paterson, 1978; Desser *et al.*, 1983 a, b; Abdel-Ghaffar *et al.*, 2004 and Abed (2005).

The accumulation of electron-dense bodies especially in regions of sutural ridge, appeared to share in the process of forming the outer shell of the spore.

The binucleated sporoplasm of the present species was similar to the most myxosporean species, (Current *et al.*, 1979; Desser *et al.*, 1983 a, b and Abed, 2005).

The electron dense inclusions in the sporoplasm of the present species were similar to that described in most myxosporean spp. (El-Matbouli *et al.*, 1990).

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