Identification Species of *Myxobolus* from Gill of *Cyprinus carpio* in East Java

(IDENTIFIKASI MYXOBOLUS SP YANG DIPEROLEH DARI INSANG IKAN KARPER DI JAWA TIMUR)

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ABSTRACT

The aim of study was to identify *Myxobolus* sp. obtained from the gills of carp (*Cyprinus carpio*) of East Java, Indonesia. The cysts containing spores were collected from the gills of carp fish. The spores were examined by wet mounts preparation, fixed with ethanol absolute solution for molecular analysis. The spores had a transparent membrane, the shell, composed of two valves. The sutural ridge running between the valves. It was two anterior polar capsules, each consisted of a coiled polar filament. An iodinophilic vacuole and sporoplasm nuclei was located in posterior part. DNA Sequences 18S rDNA followed by phylogenetic tree demonstrated that *Myxobolus* sp from Blitar was different from *Myxosoma cerebralis* of the Gene Bank. *Myxosoma cerebralis* was not found in the fresh water fish in Indonesia.

Keywords: *Myxobolus* sp, *Cyprinus carpio*.

INTRODUCTION

The first out break of *Cyprinus carpio* caused by *Myxobolus cerebralis pyriformis* infection was reported in Central Java, Indonesia, in 1951 by Sachlan (1952) and Djajadireja et al. (1982). High mortality of common carp (*C. carpio*), was caused by *Myxobolus* sp also occurred in Yogyakarta in 2002. (Kompas, 2002). Hobir (2006) found *Myxobolus cyprinii*, *Myxobolus koi*, and *Myxobolus artus* from gill of *C. carpio* from Magelang (Central Java) based on shape and size...
of spores. Little is known about the occurrence of Myxosporiases in Indonesia.

The genus of Myxobolus comprised several hundred species. Until now around 444 valid Myxobolus spp was reported from Eurasia and North America (Landsberg and Lom, 1991). The spore shell of Myxobolus ovoid or pear shaped consisted of shell embarked along the line of suture lines. It has binucleate sporoplasms, embrionic nucleus, iodino philorus or glycogen vacuole, polar capsule, and polar filament (Lom and Dykova, 1992).

Morphological classification was often difficult to determine a species of Myxobolus. Molecular analysis approach could be used to support a traditional myxozoan classification that was often confusing (Eszterbauer et al., 2001; Eszterbauer 2002).

The aim of research was to find out the species of Myxobolus and the presence of cerebralis from the fresh water fish in Indonesia.

RESEARCH METHODS

Myxozoans were collected from carp farms, Blitar, East Java. Samples were fixed in 10 % formalin solution for histological examination. Some samples were fixed in ethanol for ribosomal DNA extraction using Qiagen Dneasy kit (Qiagen Inc, Hilden), then was amplified using polymerase chain reaction (PCR) with Myx 18EF (5’- CTG GTT GAT CCT GCC AGT) and 18R (CTA CGG AAA CCT TGT TAC) primer pairs (Whipps et al., 2003). Amplification products of rDNA were purified and sequenced to compare the isolate with sequences of Myxosoma cerebralis from Gene Bank. The sequence results were aligned with Mega program, and analysed with Maximum Parsimony method using 1000x bootstrap resampling (Sourdis and Neil, 1988).

RESULTS AND DISCUSSION

Cysts were found in the gills, brain, and the eye of common carp in Blitar. The spore shell of Myxobolus was ovoid or pear shaped consisted of shell adhering together along the lines of suture lines. It has binucleate sporoplasms, embrionic nucleus, iodino philorus or glycogen vacuole, polar capsule, and polar filament (Fig. 1C).

The growth of cyst consisted of spores and infectious to other part of gills were very fast. The second lamellae of gill, was often gone and filled by cysts. The cysts were covered by a thick fibrous membrane (Fig. 1B and 1D). The other part of gill were compensated by branchiectasis on the second lamellae (Fig. 1A) or sometimes hemorrhages.

Extraction and amplification products of gill, containing Myxobolus sp from several areas in Indonesia (Muntilan, Blitar, and Bali) showed a good results (Figure 2). The length of band was about 1000bp. The results of ribosomal DNA sequences of Myxobolus sp. from Blitar were compared with Myxosoma cerebralis from Gene Bank. Their sequences were different from Myxosoma cerebralis (Figure 4).

The molecular study of Myxobolus have been done and showed 1600bp fragment of 18S rDNA region. The restriction fragment patterns of the PCR products generated by TaqI and MspI enzymes to differ M.elegans from M. hungaricus. It could be seen that each pattern was characteristic and the pattern were easily distinguishable from each other (Eszterbauer, 2002).

The sequences of Myxobolus sp from Blitar, East Java (code number 5 and 6) were clearly different from sequences of M. cerebralis. It proved that M. cerebralis have not been transmitted yet to Indonesian areas or supported the list of disease in Indonesia (BKIPM, 2010).

Whipps et al., (2004) reported that M. cerebralis was close related to Henneguya salmonicida, using 28S primers. Andree et al., (1999) have been reported about the relationship among member of the genus Myxobolus. However, two isolates of Myxobolus sp from Blitar Indonesia showed that they were very closed related to each other and one cluster with M.cerebralis using 18S primers (Figure 3 and 4).

CONCLUSIONS

The cysts of Myxobolus sp. from Blitar, East Java containing a lot of spores was covered by thick fibrous membrane. The cyst could grow fast, infectious, and metastasis as a tumour-like.
Figure 1. A. Branchiectasis on second lamellae of gill, B. Multiple cysts contained spores, *Myxobolus* sp., C. Individual spore *Myxobolus* sp. consisted of two polar capsules and iodinophilus vacuole posteriorly, D. Thick fibrous tissue of cyst membrane with many spores in the lumen. Scale bars of A, B, D 50µm; of C 20µm.

Figure 2. The extraction product of rDNA of *Myxobolus* sp. (lanes 1-7) and the amplification results of *Myxobolus* sp. (Lanes 8–14). Amplification products of *Myxobolus* sp from Blitar were at lanes 8 and 9.
Figure 3. Sequence alignment of *Myxobolus* sp. from Blitar, East Java (sequences of number A5 and A6) were compared with sequences of *Myxosoma cerebralis*, *Myxobolus koi*, and other species of *Myxobolus*. 

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Myxobolus sp. from gill and brain of common carp in Blitar was different from Myxosoma cerebralis using 18S primers. Myxosoma cerebralis was not found from the fresh water fish in Indonesia.

**SUGGESTION**

It is recommended to have sequencing DNA using primer 18S rDNA from some samples from other islands of Indonesia for further detection of the gene rDNA. Moreover, it is also important to have further study using other method such as Restriction Fragment Length Polymorphism (RFLP).

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**REFERENCES**


