PCRによるスクーチカ症の原因纖毛虫Miamiensis avidusの同定および検出

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Detection and Identification of *Miamiensis avidus* Causing Scuticociliatosis by PCR

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**ABSTRACT**—We designed a PCR primer set for specific detection of *Miamiensis avidus* causing scuticociliatosis in Japanese flounder *Paralichthys olivaceus*. In the PCR targeting the small subunit ribosomal RNA gene of *M. avidus*, the expected PCR product with 1,433 bp was amplified from four isolates of *M. avidus* including three different serotypes, but not from other scuticociliates including *Pseudocohnilembus persalini*, *P. hargisi* and *Uronema marinum*. Detection limit of the present PCR was 125 fg of genomic DNA. When a group of Japanese flounder suffering the disease was examined by the PCR, 90% of symptomatic fish and 50% of asymptomatic fish were found positive. Thus it is considered that the PCR is useful for detection and identification of *M. avidus* and consequently for diagnosis of scuticociliatosis in Japanese flounder.

**Key words:** scuticociliatosis, *Miamiensis avidus*, PCR, *Paralichthys olivaceus*, detection

Scuticociliatosis is one of the most important parasitic diseases in cultured Japanese flounder *Paralichthys olivaceus* in Asian countries such as Japan, Korea and China. This disease is characterized by signs with severe ulcer and haemorrhage in skeletal muscle, moreover scuticociliates often invade fish systemically. However, there are fish without these external signs, in which the affected tissue is limited to a brain. The causative agents of scuticociliatosis include *Uronema marinum*¹, *Philasterides dicentrarchi*², *Pseudocohnilembus persalini*³, *P. hargisi*³ and *Miamiensis avidus*⁴⁵, but it has been suggested that *M. avidus* and *Ph. dicentrarchi* are synonymously based on morphological and genetic analyses⁶⁷. In the challenge experiments to Japanese flounder using *M. avidus*, *Ps. persalini*, *Ps. hargisi* and *U. marinum*, high mortalities were only observed in the fish challenged with *M. avidus*, suggesting that the ciliate is etiologically important in the endoparasitic scuticociliatosis of Japanese flounder⁵. Moreover, it was revealed that at least three serotypes existed in Japanese isolates of *M. avidus* based on western blotting profile and immobilization assay using antisera against four *M. avidus* isolates; serotype I represented by *lyo I*, serotype II by Nakajima and serotype III by Mie0301⁸.

Currently, there has been no chemical treatment to cure endoparasitic scuticociliates. Some effective vaccines for scuticociliatosis have been developed⁹, but not commercialized in aquaculture markets. It is thus considered that rapid, sensitive and specific detection of *M. avidus* from fish at initial infection phase is important and useful to reduce economic damage. However, other scuticociliates, *Ps. persalini* and *Ps. hagisi*, existing on fish skin could make confuse detection of *M. avidus*. Thus, in the present study, a specific, sensitive and rapid PCR method for detection of *M. avidus* is newly designated and evaluated.

**Materials and Methods**

Scuticociliates

As *M. avidus*, a Korean isolate, YS¹, and three Japanese isolates, *lyo I* (serotype I), Nakajima (serotype II) and Mie0301 (serotype III)⁵, were employed in the present study. *Ps. persalini* (SCL-A)⁷ and *Ps. hargisi* (SCL-B)⁷ were provided by Dr. S. J. Jung at Chonnam National University, Korea, and *U. marinum* (WSW) was isolated from rearing seawater at the Fish Culture Center of Tottori Prefecture, Japan. These isolates were maintained in 10 mL YEHS broth (2% Lab-Lemco powder (Oxoid), 0.5% Bacto™ yeast extract (Difco), 0.5% glucose, 0.8% sodium chloride and 5% inactivated horse serum; Dr. T. Nakatsugawa, personal communication).

**Specific PCR for detection of M. avidus (M.a.-PCR)**

For designing a PCR primer set, small subunit ribosomal RNA (SSU rRNA) gene was targeted, because the target nucleotide (nt) sequences of Korean and Japanese *M. avidus* were identical to each other⁴⁸. The target gene sequence of *M. avidus* (GenBank accession number, AY550080) was compared with those of other three scuticociliates, *Ps. persalini* (AY551906), *Ps. hargisi* (AY212806) and *U. marinum* (Z22881). An *M. avidus*-specific primer set, Ma-F (5'-GTA ACT GAT CGA ATC TCT TCA C-3') and Ma-R (5'-TTC CCG TTC ACG CAA GGC CAG T-3'), was designed at 252 to 1,684 base in the SSU rRNA gene of *M. avidus*. The nt sequence of the forward primer, Ma-F (22 mer),

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Detection of M. avidus by PCR

Detection limit of M.a.-PCR

To assess the detection limit of this PCR, the genomic DNA was extracted from YS1 isolate by the method described above. An 125 ng/μL of the genomic DNA was serially diluted ten-fold with distilled water and subjected to M.a.-PCR.

Detection of M. avidus from affected fish

Detection of M. avidus was performed using Japanese flounder surviving from scuticociliatosis occurred at the Fish Culture Center of Tottori Prefecture, Japan in 2009. Each ten fish with and without scuticociliatosis signs (means body weight: 11.5 g) were collected from the same rearing tank. Abrasion and/or ulcer in the fin, muscle, mouth or outside of the gills were observed in these symptomatic fish. Brain, dorsal muscle of ocular side and gills were obtained, while epidermal mucus was collected from the part without abrasion and ulcer by a sterilized spatula. Total DNA was extracted from 50 mg of these samples using a DNeasy Blood & Tissue Kit, and subjected to the PCR.

Results and Discussion

PCR products with the expected size (1,433 bp) were amplified from genomes of all M. avidus isolates including three different serotypes, but not from genome of Ps. persalinus, Ps. hargisi or U. marinum. In our preliminary study, no difference was observed in the nucleotide sequences of SSU rRNA among Japanese and Korean M. avidus isolates including three serotypes (data not shown). Moreover, nucleotide sequences of the designed primers, Ma-F and Ma-R, showed high E value (> 0.32) to those of other parasitic SSU rRNA genes deposited in DNA database (data not shown). It is considered that M.a.-PCR is highly specific to M. avidus.

The detection limit of the M.a.-PCR was 125 fg of genomic DNA (Fig. 2). It is difficult to calculate that 125 fg of the genomic DNA correspond to how many cells' genomic DNA because recovery rate of genomic DNA is not clear. However, the obtained genomic DNA was extracted from approximately 10⁵ cells, meaning that detection limit of the preset M.a.-PCR seems to be less than a few cells of M. avidus.

Results of detection of M. avidus by M.a.-PCR from symptomatic and asymptomatic fish were shown in Table 1. In the symptomatic fish, a total detection rate of M. avidus were 90%, and those from brains, muscles, gills and epidermal mucus were 80%, 50%, 30% and 10%, respectively. These results demonstrated that detection rate of M. avidus in brain was higher than those from other tissues. It was reported that numerous scuticociliates were observed in central nervous system including meninges, telecephalon, diencephalon, optic lobes, cerebellum and medulla oblongata by histological examinations. Parama et al. detected scuticociliates in brain tissue within 15 days after experimental infections. It is thus considered that brain is the most suitable tissue for detection of M. avidus. Interestingly, detection rate of M. avidus from the epidermal mucus was lower than that of other tissues even though abrasion and/or ulcer were observed in body surfaces of the affected fish. This suggests that the epidermal mucus contains some inhibitors for PCR amplification, meaning that modification of the DNA extraction procedure from mucus seemed to be required in the future study. In one symptomatic fish exhibiting ulcer around mouth, M. avidus was not detected from any tissues (Table 1). This was hard to understand for us, but we considered that the observed ulcer could be caused by
physical damage such as friction, not by *M. avidus* infection.

In the asymptomatic fish, a total detection rate of *M. avidus* was 50%, and those from brains, muscles, gills and epidermal mucus were 40%, 20%, 20% and 0%, respectively (Table 1). It was demonstrated that the present PCR is possible to detect *M. avidus* also from asymptomatic survivor fish. Furthermore, the detection rate from brain was higher than those from other tissues. It is thus confirmed again that brain is the most suitable tissue to detect *M. avidus* by the present PCR. From these results, it is concluded that the present PCR could be useful tool for specific detection and identification of *M. avidus*, and also for diagnosis of scuticociliatosis in Japanese flounder.

**Acknowledgements**

We gratefully thank Dr. I. Hirono, Tokyo University of Marine Science and Technology, Japan, for his help in DNA sequencing.

**References**


**Table 1. Detection of *M. avidus* from Japanese flounder by *M.a*-PCR**

<table>
<thead>
<tr>
<th>Fish group</th>
<th>No</th>
<th>Organs</th>
<th>detection rate</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Brain</td>
<td>80%</td>
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<tr>
<td></td>
<td></td>
<td>Muscle</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gills</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epidermal skin mucus</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>total</td>
<td>90%</td>
</tr>
<tr>
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<tr>
<td></td>
<td>2</td>
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<td></td>
<td>5</td>
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<td>+</td>
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<tr>
<td>Asymptomatic fish (N=10)</td>
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<td>+</td>
<td>+</td>
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<td>7</td>
<td>+</td>
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<td>10</td>
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References  
ベトナム・ニャチャン湾の卵・仔魚に内部寄生する漉猟毛虫 *Ichthyodinium* sp. 感染の長期変動
A. M. Shadrin . D. S. Pavlov . M. V. Khododova

ベトナム・ニャチャン湾の卵・仔魚に内部寄生する *Ichthyodinium* sp. の感染を越季ごとに長期間に渡り調査した。寄生虫の同定は形態と 18S rRNA 錠基配列によって行った。塩基配列は臓管の線虫生産スタジアムから報告されたものと一致した。1999年に初めて検出された後、感染率は2004年までは増加し続け、その後、2007年まで高位で推移し、2008-2009年に減少したものの、2010年に再び急増した。感染率は、異なる分類群の魚種間で差異が見られた。近縁な魚種間でも大きな違いが見られた。

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ヒラメ白血球包囲化反応における顆粒球およびケモカイ

在田 修・中島篤志

ヒラメ白血球包囲化反応における顆粒球およびケモカイ

在田 修・中島篤志

PCR によるスクーターキ症の原因菌種毛虫 *Miamiensis avidus* の同定および検出

丹下菜穂子・安 準慶・北海村一真

形態観察による同定が難しいスクーターキ症原因菌種毛虫 *Miamiensis avidus* を特異的に検出するため、SSU rRNA 遺伝子を標的とした PCR 法を確立した。本 PCR の検出限界は DNA 量にして 125 fg であり、他のスクーターキ症原因菌種毛虫 3 種の DNA を增幅しなかった。感染したヒラメを PCR 検査した結果、発症魚90%、未発症魚の50%が陽性となり、特に内脏検出結果が高かった。以上より、本 PCR は *M. avidus* の同定および魚体からの迅速検出に有効であることが明らかとなった。

魚病研究, 45 (3), 115-120 (2010)

シラコより分離された *Plectosporium oratosquillae* の生体作用

在田 修・中村雅人・中村 師

シラコより分離された *Plectosporium oratosquillae* の生体作用

在田 修・中村雅人・中村 師

ベタにみられた腫瘍

E. D. Lombardi・M. Law・B. S. Lewis

米国でベタ *Betta splendens* の成魚に腫瘍がみられていた (2 例)。いずれの症例も、腫瘍巣は腎臓組織の90%以上を占めていた。病理組織学的にはこれらの腫瘍は、芽球細胞、未熟な間葉系組織および未成熟体構造を形成する上皮性腫瘍より構成されていた。また、最初の事例では囊胞性の腫瘍巣が体腔の2/3 を占め、他の症例ではよりも腫瘍巣が体腔から後部にかけて広がっていた。Wilm's Tumor-1 診断用抗体との反応性は不明瞭であった。本論文はベタにおける腫瘍の初報告である。

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16S rDNA を標的とした *Piscirickettsia salmonis* 検出用 PCR の改良

坂井貴光・谷村 明・太田祐達

16S rDNA を標的とした *Piscirickettsia salmonis* 検出用 PCR の改良

坂井貴光・谷村 明・太田祐達

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