

Studies on nontuberculous mycobacterium ;
Mycobacterium sp. pathogenic for filefish

Abstract of Doctoral Thesis

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In 2009, high levels of mortality were observed in thread-sail filefish, *Stephanolepis cirrhifer* associated with nontuberculous mycobacterial infection. From 2009 to 2013, similar infectious disease cases have been reported in both farmed and wild thread-sail filefish, and farmed black scraper (*Thamnaconus modestus*) populations in several areas of Japan, and some nontuberculous mycobacteria (abbreviated to NTM) were isolated from the diseased fish.

NTM isolates from the infected filefishes (thread-sail filefish and black scraper) were characterized using biological, biochemical analyses and antibiotics susceptibilities.

These isolates showed identical biological and biochemical characteristics and isolates showed relatively low MIC values with erythromycin and were susceptible to clarithromycin, doxycycline, and ciprofloxacin.

Transmission trials were performed to evaluate the invasion route of NTM into thread-sail filefish, and pathogenicity of the strain isolated from thread-sail filefish against black scraper.

Transmission trials were performed by immersion, peroral administration and intraperitoneally injection, however, only intraperitoneally injection could reproduce the typical features of file fish NTM infection. The results suggested that some other factors assisting NTM infection might involve in the spontaneous infection.

According to the pathogenicity test for black scraper, the strain isolated from filefish was also pathogenic to black scraper and the dead fish showed similar histopathological features to those in the thread-sail filefish.

Multi locus sequence typing, 65-kDa heat shock protein (*hsp65*) PCR restriction enzyme digestion assay (PRA) analysis and pulsed field gel electrophoresis (PFGE) were also performed.

The results of multi locus sequence typing and *hsp65* PRA pattern analysis suggested that the NTM isolated from filefish is a novel specie and *Hae*III digestion could be used to distinguish NJB0901 from other type strains.

The result of PFGE analysis showed all the strains isolated from different conditions have same genotypic character.

Protein profiling and lipid profiling were performed by MALDI-TOF MS. Protein profiling using MALDI Biotyper 3.1 (Bruker Daltonics, Inc.) was performed with filefish strain NJB0901, *M. chelonae* JCM6388^T, and *M. salmoniphilum* ATCC13758^T.

Acquired results suggested that NJB0901 have unique protein profile. MALDI-TOF MS analysis of total lipids showed that filefish strain NJB0901 possessed unique metabolic mechanism for Tween 80.

PCR primers set was designed for specific detection of *Mycobacterium* sp. isolated from filefish, and evaluated the availability for rapid diagnosis. The designed primers were specifically reacted with *Mycobacterium* sp. isolated from filefish and did not react with other NTM type strains. This result indicated high availability of this PCR primers set for rapid detection of filefish NTM pathogen.