

Studies on transmission cycles of *Mycobacterium marinum*
in closed-rearing environment of aquaria in Japan

Abstract of Doctoral Thesis

Takeshi Komine

Graduate School of Veterinary Medicine and Life Science

Nippon Veterinary and Life Science University

Mycobacterium marinum is a nontuberculous mycobacterium (NTM), and causes infectious disease in mammals including humans, fish, amphibians, reptiles, and arthropods. *M. marinum* is widely distributed in nature, especially in the aquatic environment. Additionally, this pathogen has been also isolated from aquatic life such as mollusks, echinoderms, and amoebae. Therefore, there is a hypothesis that transmission of this pathogen includes a variety of animals and environmental factors. However, that has not been proven sufficiently yet. The present study aims to confirm the hypothesis about transmission of *M. marinum* in closed-rearing environment using molecular epidemiological methods (core single nucleotide polymorphism (SNP) analysis). In addition, to evaluate usefulness of variable number of tandem repeats (VNTR) analysis as a rapid method, a comparison of VNTR and core SNP analyses was conducted. We diagnosed *M. marinum* infection and performed isolation of this pathogen from animals and environmental components in two aquaria (aquarium A and B) in Japan. In aquarium A, *M. marinum* infection was diagnosed in a tank rearing flyingfish and a tank exhibiting eelgrasses (*Zostera marina*) bed, and the causative strains were also isolated from fish and filter sand in both the tanks. In aquarium B, it was revealed that the infection occurred in some fish reared in a tank exhibiting eelgrasses (*Zostera marina*) bed, and *M. marinum* strains were isolated from fish, invertebrates, and environmental components in the tank. Core SNP analysis classified isolates from the aquaria into four clusters. In aquarium A, isolates from flyingfish and filter sand in the tanks rearing flyingfish formed a cluster. In addition, isolates from wrasse and bottom sand in the tank exhibiting eelgrasses bed formed another cluster. For aquarium B, isolates from fish, invertebrates and environmental components in the tank exhibiting eelgrasses bed formed another cluster. Hence, it was suggested that fish and environmental components could be involved in the transmissions of *M. marinum* in aquarium A, and fish, invertebrates and environmental components could be involved in the transmission of it in aquarium B. By using VNTR analysis, the genotypes of most isolates were generally classified into three groups, however this method couldn't discriminate some strains obtained at aquarium A and aquarium B, which were geographically isolated. While we conducted the second VNTR analysis with additional VNTR loci which have been previously reported and were newly generated to improve the resolving ability, the analysis still couldn't discriminate those

isolates. In this study, to confirm transmission cycles of *M. marinum*, we conducted the diagnosis of *M. marinum* infection, isolation of this pathogen from various factors, and the phylogenetic analysis in obtained isolates. The comparison of two genotyping methods was also performed. This molecular-based study epidemiologically confirmed that various factors including fish, invertebrates and environmental components can be involved in transmission cycles of *M. marinum*. Furthermore, it was conducted that further investigation of VNTR locus sets is needed to utilize VNTR analysis.