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Identifying Pathogenic Mycobacterium Species Using Multiple Gene Phylogenetic Analysis

Authors : Lemar Blake, Chris Oura, Ayanna C. N. Phillips Savage

Abstract : Improved DNA sequencing technology has greatly enhanced bacterial identification, especially for organisms that are difficult to culture. Mycobacteriosis with consistent hyphema, bilateral exophthalmia, open mouth gape and ocular lesions, were observed in various fish populations at the School of Veterinary Medicine, Aquaculture/Aquatic Animal Health Unit. Objective: To identify the species of Mycobacterium that is affecting aquarium fish at the School of Veterinary Medicine, Aquaculture/Aquatic Animal Health Unit. Method: A total of 13 fish samples were collected and analyzed via: Ziehl-Neelsen, conventional polymerase chain reaction (PCR) and real-time PCR. These tests were carried out simultaneously for confirmation. The following combination of conventional primers: 16s rRNA (564 bp), rpoB (396 bp), sod (408 bp) were used. Concatenation of the gene fragments was carried out to phylogenetically classify the organism. Results: Acid fast non-branching bacilli were detected in all samples from homogenized internal organs. All 13 acid fast samples were positive for Mycobacterium via real-time PCR. Partial gene sequences using all three primer sets were obtained from two samples and demonstrated a novel strain. A strain 99% related to Mycobacterium marinum was also confirmed in one sample, using 16srRNA and rpoB genes. The two novel strains were clustered with the rapid growers and strains that are known to affect humans. Conclusions: Phylogenetic analysis demonstrated two novel Mycobacterium strains with the potential of being zoonotic and one strain 99% related to Mycobacterium marinum.

Keywords : polymerase chain reaction, phylogenetic, DNA sequencing, zoonotic
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