Genomic Insights into the Fish-Pathogenic *Mycobacterium pseudoshottsii* Strain AR Recovered from Meagre (*Argyrosomus regius*)

Panagiota Stathopoulou,a Elias Asimakis,a Yiannis Petropoulos,a Georgia Apostolopoulou,a George Tsiamisa

aDepartment of Environmental Engineering, University of Patras, Agrinio, Greece

**ABSTRACT**

Mycobacteriosis can cause morbidity and mortality and is characterized as a disease with zoonotic potential that is found worldwide in both fresh and marine fish. Here, we report the draft genome sequence of *Mycobacterium pseudoshottsii* strain AR, an isolate recovered from infected meagre (*Argyrosomus regius*) raised on farms in Greece.

The meagre (*Argyrosomus regius*) is considered to be one of the best potential candidates for large-scale fish farming in the Mediterranean (1). Although global meagre production is reported to have increased rapidly, one of the most important bottlenecks remains systemic granulomatosis (2). The etiology of the disease has been related to nocardiosis in one incident from Greece (3), while two cases of mycobacteriosis have been reported from Turkey (4, 5). Here, we report on the genomic analysis of a granuloma-associated *Mycobacterium* strain isolated from infected meagre raised on farms in Greece.

In late 2019, meagre showing lethargy, anorexia, abdominal swimming, and enlarged internal organs were recorded in fish farms. Pathogen isolation was attempted, with the affected spleens exhibiting white nodules. The tissues were aseptically dissected, decontaminated, homogenized (6), and inoculated onto Middlebrook 7H10 agar (7). Incubation was performed at 30°C for 2 months. The first isolate grew 6 weeks later. The colonies were rough, not emulsifiable in water, and unable to grow at 37°C or below 20°C.

Genomic DNA was extracted from a single colony using lysis buffer containing lysozyme (Sigma-Aldrich), according to Haught et al. (8). DNA was quantified in triplicates with the Quant-iT double-stranded DNA assay (Invitrogen) in an Eppendorf AF2200 plate reader. The genomic library was prepared using the Nextera XT library preparation kit (Illumina, San Diego, CA) according to the manufacturer’s protocol. The library was quantified using the Kapa Biosystems kit for Illumina on a Roche LightCycler 96 quantitative PCR system. The library was sequenced with 30-fold coverage on the Illumina HiSeq platform using a 250-bp paired-end protocol, producing 1,541,863 reads. Reads were adapter trimmed using Trimmomatic v. 0.30, with a sliding window quality score cutoff value of Q15 (9). FastQC v. 0.11.8 was used to check the quality of the validated reads (10). Reads were de novo assembled into 298 contigs using SPAdes v. 3.14 (11). The quality of the assembly was evaluated with Quast v. 5.0.2 (12). Taxonomic assignment of the reads was performed using Kraken v. 2.0.8 (13), and the RAST algorithm v. 2.0 (14–16) was used for genome annotation.

The assembled draft genome had a length of 5,934,315 bp, with a GC content of 65.6%. The largest contig was 162,927 bp long, the L50 value was 46, and the N50 value was 40,009 bp. Most of the reads were classified into the genus *Mycobacterium*. The draft genome contained 6,113 unique predicted genes.

Phylogenomic analysis based on the multiple alignment with MUSCLE (17) of the concatenated sequences (53 kbp in length) of 52 single copy genes (18) against the concatenated sequences of the same genes extracted from other known *Mycobacterium*...
ACKNOWLEDGMENTS

This work was cofunded by Greece and the European Union through the Fisheries and Maritime Operational Program 2014–2020 managed by the Hellenic Ministry of Rural Development and Food in the context of the project Study of the Genome and Microbial Communities in the Development and Production of Aquacultured Sea Bream and Sea Bass (MIS-5010952). This research was also cofinanced by Greece and the European Union (European Social Fund [ESF]) through the Operational Program Human Resources Development, Education and Lifelong Learning in the context of the project Reinforcement of Postdoctoral Researchers—2nd Cycle (MIS-5033021), implemented by the State Scholarships Foundation (IKY).

REFERENCES


