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Draft Genome Sequence of *Mycobacterium abscessus* Treated with a Fluoroquinolone in a Time-Dependent Manner

Du-Gyeong Han, Ji-A Jeong, Sung-Kyoung Lee, Seong-Han Kim, and Se-Mi Jeon*

Department of Bacterial Disease Research, Center for Infectious Disease Research, National Institute of Infectious Disease, Korea National Institute of Health, Korea Disease Control and Prevention Agency, Cheongju 28159, Republic of Korea

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This study aimed to confirm the induction of resistance to other drug classes by treating *Mycobacterium abscessus* with moxifloxacin, a fluoroquinolone used for treating nontuberculous mycobacteria infection, and to obtain genetic data for improving treatment. The reads were assembled and analyzed using reference strain sequence data, and the whole-genome and transcriptome sequences of four strains (MD2, MD4, MD6, and MD8) were reported. Antibiotic resistance was not induced by moxifloxacin treatment; however, transcriptomic analysis revealed that the expression of genes responding to stress was upregulated.

Keywords: ***Mycobacterium abscessus*, genome, transcriptome, moxifloxacin, Fluoroquinolone, resistance**

Nontuberculous mycobacteria (NTM) comprise more than 180 species of mycobacteria, excluding *Mycobacterium tuberculosis* and *M. leprae*, and are widely distributed in the natural environment, mainly in water and soil [1]. NTM can be classified into two groups based on their growth time: slowly growing bacteria such as *M. avium* and *M. intracellulare*, which require more than 7 days, and rapidly growing mycobacteria such as *M. abscessus* and *M. massiliense*, which require less than 7 days [2]. NTM mainly infect the respiratory tract and cause lung disease in humans and are treated with a combination of antibiotics, including aminoglycosides, macrolides, and fluoroquinolones [3]. The prevalence of NTM diseases has been increasing in the Republic of Korea, although that of tuberculosis has been decreasing [4, 5]. *M. abscessus* was reclassified as *Mycobacteroides* by Gupta in 2018 [6]; however, it is still used with *Mycobacterium*. It has high innate resistance to multiple antibiotics, thus making treatment difficult with poor treatment outcomes. In this study, we treated *M. abscessus* with moxifloxacin (MXF), a fluoroquinolone used for treating NTM infection, for a certain time period to confirm possible resistance to other classes of antibiotics and to obtain a genetic resource for improving NTM infection treatment.

M. abscessus ATCC 19977 was cultured in 7H9 broth (BD, USA) supplemented with 0.2% glycerol (Sigma-Aldrich, USA) and 0.05% Tween-80 (Sigma-Aldrich). The minimal inhibitory concentration (MIC) of MXF (Sigma-Aldrich) was determined using the broth microdilution method according to the clinical and laboratory standard institute guideline [7] and was confirmed to be 64 μg/ml. For the kill curve assay, a culture of the log-phase strain with an OD_{600} value of 0.75–0.85 was treated with 640 μg/ml MXF, which is 10-fold MIC, for 8 days, and the samples were harvested on day 2, 4, 6,

***Corresponding author**
Phone: +82-43-719-8462, Fax: +82-43-719-8489
E-mail: sem21c@korea.kr

and 8.

Genomic DNA was isolated using Quick-DNA Fungal/Bacterial Kits (Zymo, USA), and the size and quantity of DNA were determined using Genomic DNA ScreenTape Analysis (Agilent, USA). A DNA library was constructed using the TruSeq Nano DNA Kit (Illumina, USA), and the library quality was assessed using DNA ScreenTape Analysis. To generate sequence data from the validated library, the Hiseq X ten Reagent Kit version 2.5 (Illumina) and the Hiseq X ten instrument (Illumina) were used to obtain paired-end reads of 2 × 150 bp. The reads were trimmed to remove low-quality reads and indices, and reference assembly and variant calling analysis were performed using assembly data of the reference strain (GCF_000069185.1). All analyses related to the produced whole-genome data were performed using default parameters in CLC Genomics Workbench version 22.0 (Qiagen, USA).

Total RNA was isolated using the RNeasy® Plus Mini Kit (Qiagen) with Lysing Matrix B (MP biomedicals, USA), and residual DNA was removed by DNase treatment. The quantity and purity of the sample were determined using RNA ScreenTape Analysis (Agilent), and the ribosomal RNA amount in the sample was minimized using NEBNext® rRNA Depletion Kit (New England Biolabs, USA). Complementary DNA library was synthesized using the TruSeq Stranded Total RNA Library Prep Gold Kit (Illumina), and the quality of the produced library was assessed using DNA ScreenTape Analysis. Paired-end reads of 2 × 100 bp were generated using the NovaSeq 6000 S4 Reagent Kit version 1.5 (Illumina) and the NovaSeq 6000 instrument (Illumina). Low-quality reads and indices were removed from the data via the trimming process. RNAseq data were obtained using the track data extracted from the reference strain assembly data (GCF_000069185.1), and normalization and differentially expressed gene (DEG) analysis were performed according to the conditions set during metadata creation. All analyses related to the produced transcriptome data were performed using default parameters in CLC Genomics Workbench version 22.0 (Qiagen). Additionally, gene ontology analysis was conducted using the DAVID functional annotation analysis tool (https://david.ncifcrf.gov, National Institutes of Health, USA) and Prism version 10 (GraphPad, USA).

Whole-genome sequencing analysis revealed that 7,721,354 reads (total 1,154,129,994 bp, strain MD2), 7,017,030 reads (total 1,045,990,824 bp, strain MD4), 8,373,691 reads (total 1,247,466,370 bp, strain MD6), and 8,229,967 reads (total 1,224,067,405 bp, strain MD8) were acquired, and genome coverage was confirmed to be 225.72, 205.28, 244.79, and 238.12, respectively. The genetic stats (Table 1) of the four strains were mostly similar; however, the GC content of strain MD8, harvested at the latest time point, was relatively low compared with that of the other strain. Based on the variant calling analysis results, no significant mutations

Table 1. Genetic characteristics of *Mycobacterium abscessus* strain MD2, MD4, MD6, and MD8.

Strain		MD2	MD4	MD6	MD8
SRA accession No.	[T*]	SRR26821764	SRR26821762	SRR26821760	SRR26821758
	[G*]	SRR26821765	SRR26821763	SRR26821761	SRR26821759
GenBank accession No.	[C*]	CP142119	CP142117	CP142115	CP142113
	[P*]	CP142120	CP142118	CP142116	CP142114
Genome size (bp)		5,079,890	5,079,889	5,079,889	5,079,890
GC contents (%)		64.13	64.22	64.18	62.37
Contig		2	2	2	2
Plasmid (bp)		1 (23,319)	1 (23,319)	1 (23,319)	1 (23,319)
Total genes		5025	5025	5025	5025
CDSs		4972	4972	4972	4972
rRNA		3	3	3	3
tRNA		47	47	47	47
ncRNA		2	2	2	2

*T, transcriptome; G, genome; C, chromosome; P, plasmid

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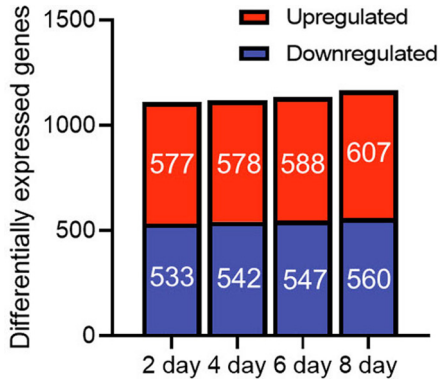

Fig. 1. Gene expression profile of *Mycobacterium abscessus* treated with MXF based on DEG analysis in a time-dependent manner.

were detected in resistance genes such as *gyrA* and *gyrB* [8].

As a result of analyzing gene expression patterns during the cultivation period after MXF treatment using transcriptomic data (Fig. 1), the number of genes whose trends changed over time gradually increased. The largest change was confirmed on day 8; however, there was no significant difference from other time points. In the common DEG analysis conducted to confirm gene expression trends according to antibiotic treatment (Fig. 2), there were more genes whose expression was upregulated than those whose expression was downregulated. Based on the common DEG analysis results, the functional distribution of the genes was analyzed (Fig. 2), and it was confirmed that genes related to DNA integration, transcription factor activity, sequence-specific DNA binding, oxidoreductase activity, and DNA binding were distributed in the gene group with upregulated expression, and genes related to fatty acid biosynthetic process, transferase activity, transferring acyl groups, FAD binding, biotin carboxylase activity, and metal ion binding were distributed in the gene group with downregulated expression.

In conclusion, we found that *M. abscessus* gene expression patterns changed in response to stress, although no major changes were observed in the DNA sequence. The study findings may be helpful for future research on improving antibiotic treatment and resistance.

Acknowledgments

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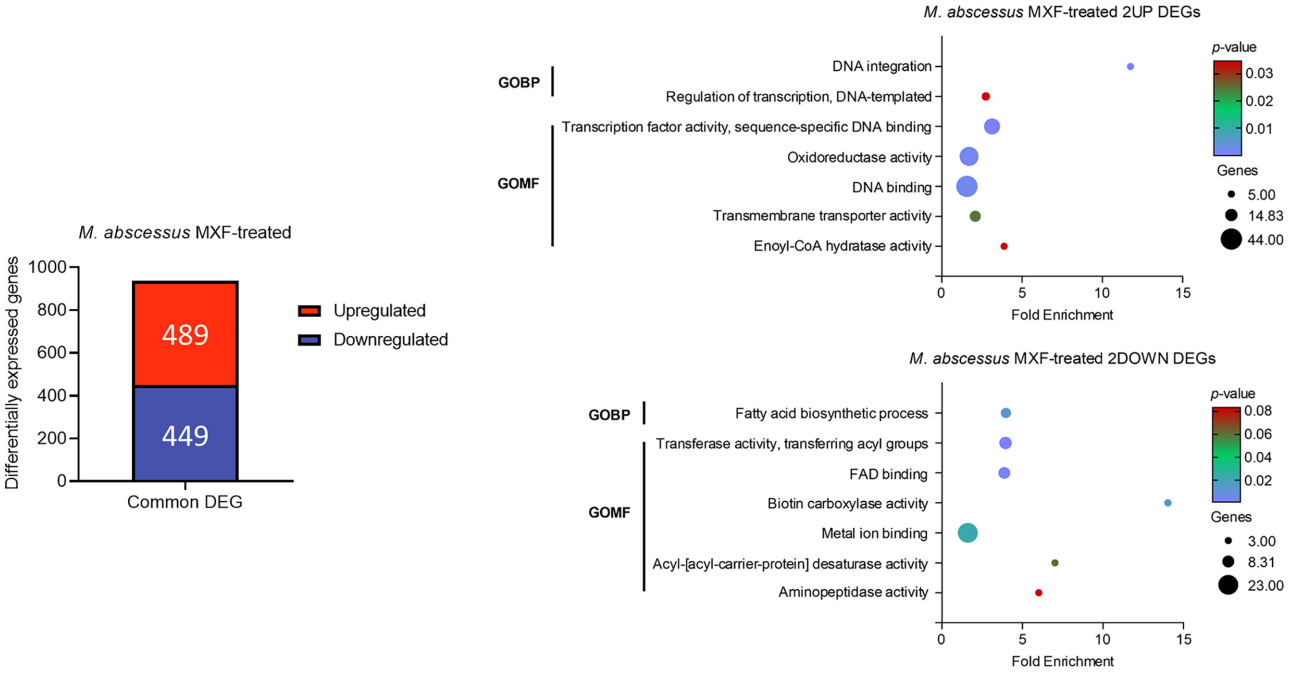

Fig. 2. Gene expression profile and functional distribution (GOBP, Gene Ontology Biological Process; GOMF, Gene Ontology Molecular Function) of genes in *Mycobacterium abscessus* treated with MXF based on common DEG analysis.

Conflicts of Interest

The authors have no financial conflicts of interest to declare.

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