

## Title: Exploring the Genetic Basis of Biofilm-Driven Antibiotic Tolerance in *Mycobacterium marinum*

### Authors:

Emilia Lintilä, Nelli Vahvelainen, Jesse Anttila, Erika Niininen, Laura Kantanen, Milka Hammarén, Matalleena Parikka

### Keywords:

Antibiotic tolerance, Biofilms, Mycobacteria, Tuberculosis

### Abstract

Tuberculosis is an infectious disease that caused over 1 million deaths in 2023. The causative agent of the disease, *Mycobacterium tuberculosis* forms biofilms *in vitro* and *in vivo*, which likely contributes to antibiotic-tolerant phenotype of mycobacteria. Thus, we aim to find and characterize mutants with biofilm defects to understand the role of biofilms and ECM components during tuberculosis infection. The goal of this study is to discover regulatory mechanisms underlying biofilm formation and drug tolerance of mycobacteria.

The study is carried out with *Mycobacterium marinum* which is closely related to *M. tuberculosis*. We aim to screen 18,000  $\phi$ MycoMar T7 transposon mutant clones on Congo red agar plates to identify mutants with different color or colony morphology from wild type *M. marinum*. The Congo red dye binds to ECM components such as amyloids and cellulose. Currently, 4200 mutants have been screened, and 158 potential hits with red and/or rough or smooth morphology have been identified. The biomass per cell of the hit clones was quantified by crystal violet staining. 55% of the mutants had significantly different biomass from the wild type. Mutants with biofilm-defect were screened for antibiotic tolerance. Among the hits identified so far, 21% of mutants were significantly less tolerant to a common first-line antibiotic, rifampicin, than the wild type *M. marinum*. Next, the most interesting mutants will be sequenced, further characterized using proteomics, and tested for virulence and tolerance *in vivo* in the adult zebrafish tuberculosis model. Altogether, this screen will provide insight into biofilm-associated tolerance in mycobacteria.