Title: Functional analysis of potential DNA repair proteins – Msmeg_1891 and Rv3226c – in *Mycobacterium* genus.

Abstract

Mycobacterium species are constantly exposed to genotoxic agents, leading to DNA damage. For this reason, their cells require the presence of functional proteins involved in the repair of genetic material. One of the relatively recently identified proteins of unknown function, presumably involved in such repairs, is Msmeg_1891 (M. smegmatis) and its homologue Rv3226c (M. tuberculosis) that belong to the SOS response family (SRAP) (Płociński et al., 2017; Płociński et al., 2019).

The aim of this doctoral thesis was the functional analysis of potential DNA repair proteins - Msmeg_1891 and Rv3226c - in mycobacteria of the genus Mycobacterium. In the first stage, the construction of M. smegmatis deletion mutants and M. tuberculosis, with deletions within the msmeg_1891 and rv3226c genes, as well as multiple mutants of M. smegmatis, devoid of functional repair proteins, involved, e.g., in BER or NHEJ and additionally the Msmeg_1891 protein. A strain with reduced expression of the msmeg_1891 gene was also constructed using the CRISPRi/dCas9 method. Then, all obtained mutants were subjected to phenotypic analyzes after DNA damage caused by selected genotoxic factors, i.e. MMC, UV radiation, H2O2, CHP and MMS. The analyzes carried out allowed to observe the sensitization of strains lacking Rv3226c and Msmeg_1891 proteins (or with its reduced level) after exposure to 0.4% MMS. In the next step, purification of the recombinant Msmeg_1891 protein in fusion with a polyhistidine tag (HIS-tag) was started in the heterologous systems of E. coli BL21 and E. coli Rosetta. For this purpose, metal affinity chromatography on a nickel deposit and high-performance liquid chromatography AKTA Start were used. Obtaining the Msmeg_1891 preparations was a milestone of this work, due to their use in the further part of the work. After obtaining the Msmeg_1891 protein, the preparation of polyvalent antiserum containing anti-Msmeg_1891 antibodies was started (the serum was obtained in cooperation with Dr. Bożena Dziadek, professor of the University of Lodz, Department of Molecular Microbiology, University of Lodz). The antibodies were then purified to an experimental titer of 1:3200. In the next step, the ability of Msmeg_1891 to bind single-stranded DNA (ssDNA) and RNA substrates was demonstrated using the electrophoretic

mobility shift assay (EMSA). The activity of the Msmeg_1891 protein was also determined, which consists of the cleavage of 2'-deoxyuridine from the double-stranded DNA substrate, in a manner like that of uracil-DNA glycosylase (Udg). Moreover, the binding strength of Msmeg_1891 to single-stranded substrates containing (or not) was verified using microscale thermophoresis. Stronger binding of Msmeg_1891 to the substrate was demonstrated, along with an increase in the number of 2'-deoxyuridines. To specify the repair pathway in which the tested protein could participate, partner proteins were searched using Msmeg_1891 as bait, which allowed the selection of Msmeg_3883 (DNA 5'-3' exonuclease) analogous to the exonuclease domain of the PolA protein, Msmeg_0866 (DNA or RNA helicase) or Msmeg_4912 (DinG, 5'-3' helicase) as potential partners for the protein under study. In the next stage of the work, the expression level of msmeg_1891 was analysed, which was 1000 times higher after exposure of M. smegmatis cultures to MMS. The Msmeg_1891 protein level was also significantly higher, which was confirmed using western blot and anti-Msmeg_1891 antibodies. To determine the variability of the transcript levels of specific genes in M. tuberculosis Δrv3226c cells after MMS treatment, a global transcriptome analysis after MMS treatment with this compound was performed, which showed a significant response of the MMS treated strains, however, the difference between the M. tuberculosis Δrv3226c strain and the control (wild type strain) was relatively small.

The studies carried out allowed for precise characterization of the Msmeg_1891 (Rv3226c) protein by observing the sensitization of mutants lacking msmeg_1891/rv3226c (and strain with a reduced level of Msmeg_1891 protein) to MMS, suggesting the participation of these proteins in the repair of DNA damage resulting from the methylation of nitrogenous bases. In addition, Msmeg_1891 was shown to be able to bind to DNA and RNA substrates, as well as the possibility of cutting 2'-deoxyuridine from the double-stranded DNA substrate, which may indicate the similarity of the proteins studied in this work to the uracil-DNA glycosylase protein.