Mycobacterium tuberculosis, the human super-pathogen and etiologic agent of tuberculosis, has claimed around 1,5mlns of lives in the year of 2017, the World Health Organization reports. It currently infects nearly two billion people worldwide and is considered the most serious bacterial threat to the global health care system. Treating tuberculosis is challenging as it requires prolonged medication with combination of four to five mycobacteria-specific antibiotics. Increasing rates of incidence of drug-resistant and multidrug-resistant tuberculosis is also alarming.

Unrepaired discontinuity of a single or both DNA strands may be lethal to a microorganism as it may result in mutations or genome fragmentation and thus, a complete loss of viability. Mycobacteria has evolved a series of repair pathways with clear potential relevance to pathogenesis. In response to *M. tuberculosis* infection, human macrophages become induced and produce significant amounts of reactive oxygen and nitrogen species, which are known to cause DNA damage, primarily oxidation of guanines and denitrification of nucleobases. *M. tuberculosis* can survive or even thrive in human macrophages due to various protective mechanisms and virulence factors and it possesses a vast battery of DNA repair systems, with emphasized base excision repair (BER) and at least three distinct pathways involved in double-strand break (DSB) repairs.

Recent, high-impact studies have identified interesting new features of the DNA repair machinery in mycobacteria. The projects' principal investigator was personally involved in the discovery of the NucS-driven, archaea-like mismatch repair [Castaneda-Garcia et al. *Nature Communications, 2017*] and the initial characterization of stationary phase BER pathway involving archaeo-eukaryotic primase-polymerase – Prim-PolC [Plocinski et al. *Nature Communications, 2017*].

The current study is set to investigate in details, the mechanisms of repair of DSBs as well as nicks and single nucleotide gaps in the DNA, using mycobacterial models. **Firstly, to better characterize the DNA break repair mechanisms during the exponential and stationary phases of growth and to understand how are they selected. Specifically, to investigate the roles of yet uncharacterized or vaguely characterized DNA damage sensing and processing factors, found in our previous studies.** Microbiology, biochemistry and molecular biology related methods will be used during experimentation. The project implies the extensive use of proteomics, including the identification of DNA-protein and protein-protein complexes by mass spectrometry analysis. Stationary phase-related DNA break repairs are known to introduce ribonucleotides into the genetic material during the initial repair. These primary repair products pose as potential instabilities and require further processing by ribonucleotide excision repair system, not yet described to operate in mycobacteria. **Our second goal is to investigate the molecular mechanisms via which the ribonucleotide-containing DNA repair products are processed to fully restore the genomic integrity in acid fast bacteria.** The study will exploit next generation DNA sequencing (Nanopore and Illumina technologies) of the mycobacterial strains after exposing them to oxidizing genotoxins to study the repair processes in real time and to report the timing and the extent of ribonucleotide insertions during DNA break repairs.

The proposed project involves extensive studies of mechanisms of DNA break repairs, that are essentially conserved in eukaryotes and bacteria. On one hand, the project will focus on conserved mechanisms and its findings could be extrapolated towards better understanding of human DNA repair, relevant to carcinogenesis, aging an many other processes. On the other hand, finding differences in studied here important DNA break repair pathways may help to identify essential processes and enzymatic activities that may be exploited for targeted inhibition of bacteria as part of anti-tuberculosis chemotherapy.