Infectious diseases are one of the leading causes of death worldwide. Among the main culprits of nosocomial infections are enterococci. Within the Enterococcus genus, *E. faecium* is currently the most therapeutically challenging organism. It belongs to the so-called ESKAPE pathogens, i.e., microorganisms that can "escape" most available antimicrobial treatments. Clinical isolates of this bacterium are resistant to many antibiotics, including vancomycin, which is often the drug of last resort. Interestingly, it turns out that the determinants of resistance to this antibiotic are found almost exclusively on pRUM-type plasmids, which additionally possess the toxin-antitoxin (TA) *axe-txe* system. Plasmids are one of the key elements responsible for the spread of antibiotic resistance. The TA systems localized on them account for stable persistence of these genetic elements in the bacterial population and can influence various physiological processes in the host cells, including those related to virulence and pathogenesis. The toxin-antitoxin cassettes are small modules encoding a toxin and its specific antidote. The toxin is often referred to as an intracellular molecular bomb, since its release from the complex with the antitoxin results in inhibition of cell growth or even cell death. Hence, precise and tight regulation of expression of both genes is essential for cell safety.

Genes of a typical type II toxin-antitoxin operon, which the *axe-txe* system belongs to, are usually straightforwardly controlled by negative autoregulation. However, our previous studies have identified several different genetic components within this operon, such as additional promoters, an antisense transcript, a termination hairpin or a mini-gene, each of which is important for proper function of the entire cassette. Such a variety of regulatory elements definitely distinguishes this system from other TA modules known so far. The experiments planned in the first part of this project are aimed at understanding the exact mechanism of *axe-txe* gene expression regulation and the interplay of all existing components of this complex system, which precisely tunes the ratio of Axe antitoxin to Txe toxin in its natural host, i.e. the *E. faecium* cells. For this purpose we are going to employ diverse *in vivo* and *in vitro* techniques, like reporter gene fusions, Northern blot and RT-qPCR analysis, EMSA and *in vitro* transcription studies, ribonuclease cleavage, as well as toeprinting assays. In addition, the role of the *axe-txe* cassette in enterococcal cell function has not yet been investigated. Therefore, the experiments planned in the second part of this project aim to test whether the presence of this module on the plasmid has any effect on the efficiency of bacteriophage infectivity, biofilm formation, as well as on the ability of the enterococcal cells to persist at different types of stress conditions. Finally, we want to assess the capability of the Axe antitoxin to regulate the host’s genes by performing transcriptomic analyses.

We expect that the information obtained from this proposal implementation will significantly expand the existing knowledge of the various strategies and mechanisms used by bacteria to precisely regulate gene expression, including genes of the toxin-antitoxin systems. Due to the prevalence of TA modules in clinically important bacterial strains, it is believed that artificial activation of a toxin may be an innovative strategy in the fight against pathogens. Moreover, the toxin-antitoxin systems have great potential as tools for many other practical applications in medicine and biotechnology. Thus, learning about the effects of these modules on bacterial cell function and the mechanisms of tight regulation of expression of both genes is crucial for future implementations to be efficient and safe.