

*Clostridioides difficile* infections (CDI) are among the most serious healthcare-associated infections, particularly affecting vulnerable patients, such as those with inflammatory bowel diseases (IBD) like Crohn's disease. Individuals with Crohn's disease often suffer from gut microbiota dysbiosis, which weakens the gut's natural defenses and favors pathogenic bacteria like *C. difficile*. Moreover, standard antibiotic therapies can paradoxically worsen dysbiosis, contributing to high rates of infection recurrence and treatment failure. Thus, there is an urgent need for alternative, safer, and microbiota-friendly therapeutic approaches.

The proposed project explores the potential of natural bioactive compounds – polyphenols (quercetin, resveratrol, epigallocatechin gallate) – and metabolites produced by potentially probiotic gut bacteria (*Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, *Bacteroides fragilis*) to counteract *C. difficile* virulence and modulate gut microbiota toward a healthier composition. Laboratory experiments will be performed using a panel of clinical *C. difficile* strains, representing different ribotypes, and gut microbiota samples obtained from a patient with Crohn's disease affecting the colon. The research plan involves assessing the effect of tested substances on bacterial growth, toxin production, adhesion to intestinal cell lines, sporulation, motility, and biofilm formation. In addition, studies of gut microbiota modulation using fermentation models that simulate conditions present in the digestive tract are planned. Analytical techniques, including next-generation sequencing (NGS) and high-performance liquid chromatography (HPLC), will be employed to study changes in microbiota composition and short-, and branched chain fatty acid (SCFA/BCFA) production.

This project addresses a crucial gap in current therapies by proposing new microbiota-based strategies using natural, well-tolerated compounds. Its results could significantly enhance our understanding of the interactions between diet, microbiota, and pathogenic bacteria, and open the door for developing adjunct therapies for CDI and Crohn's disease. In the long term, this research could contribute to new nutraceutical approaches supporting gut health and reducing the burden of recurrent intestinal infections.