With today's fast-paced lifestyle, people are constantly exposed to multiple negative factors, such as an unbalanced diet rich in highly processed food products, drug abuse, chronic stress and dysbiosis. These factors can instigate a cascade of prolonged inflammation and oxidative stress, leading to the development of various disorders. And at the center of the battleground for homeostasis is the intestinal barrier. Imagine the intestinal barrier as a fortress, standing guard against invaders while selectively allowing nutrients to pass through. However, in the face of constant attack by factors resulting from our modern lifestyle the fortress weakens, leaving the human body vulnerable to a myriad of health issues. The disruption of the intestinal barrier is now widely acknowledged as a pivotal factor contributing to a diverse spectrum of diseases, ranging from inflammatory bowel disease and irritable bowel syndrome to conditions like celiac disease, cancer, autism, chronic fatigue syndrome, neurodegenerative disorders, and even COVID-19. Consequently, there is a growing focus on exploring dietary supplements that could potentially prevent or alleviate disorders linked to inflammation-induced intestinal barrier disruption, offering promise for improved patient outcomes and overall well-being. Probiotics are of particular interest as they are considered a potential treatment for fortifying the intestinal barrier. This is noteworthy given the significant influence of the gut microbiota on modulating the function and structure of the intestinal barrier. The most widely used and studied probiotic bacteria include representatives from lactobacilli that belong to the lactic acid bacteria (LAB) group. As a member of the LAB group, *Lactococcus* genus comprises an important pool of strains that can benefit the host intestinal barrier function. Widely used in dairy starter cultures, lactococci demonstrate diverse health-promoting properties, including antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory effects. This highlights the potential application of *Lactococcus* strains in the development of new functional foods with therapeutic benefits. However, despite the potential benefits, research on the probiotic properties of *Lactococcus* and its impact on intestinal barrier function is limited, and even less is known about the mechanisms by which they can protect the intestinal barrier against inflammation-induced damage. Only few studies have examined how specific *Lactococcus* strains affect the intestinal barrier and modulate resident microbiota composition. Therefore, exploring the probiotic potential of *Lactococcus* strains is essential to elucidate their ability to counteract inflammation-induced intestinal barrier disruption, which could pave the way for targeted interventions aimed at preserving intestinal integrity and managing gastrointestinal disorders more effectively.

Objectives: The project aims to evaluate the efficacy of *Lactococcus* strains in mitigating inflammation-induced disruption of the intestinal barrier. The end goal is to identify the most promising *Lactococcus* strain with well-characterized gut barrier protective properties that could potentially be used as a probiotic. Additionally, the project focuses on elucidating the underlying mechanisms by which lactococci can enhance intestinal barrier function.

Research plan: In the first stage of the project, ten *Lactococcus* strains that can produce health-promoting metabolites will be tested for the ability to improve the cell viability of intestinal epithelial cells (Caco-2 cell line) after inflammation-induced damage. The five most promising strains will be selected for further evaluation of their impact on intestinal permeability and integrity using an *in vitro* model that incorporates two key cell types relevant to intestinal inflammation: intestinal epithelial cells and immune cells (THP-1 cell line). In the next stage, the effects of *Lactococcus* treatment on the secretion of inflammatory cytokines, the expression of a wide range of inflammatory markers, elements of the antioxidant defense system and key protein markers of the intestinal integrity will be tested. Utilizing immunofluorescence analysis coupled with confocal microscopy one selected *Lactococcus* strain will be tested for the ability to modulate the morphology of tight junctions, which play a pivotal role in maintaining proper intestinal barrier function.

Substantial results expected: We expect that our findings will significantly contribute to our understanding of the biological activity of *Lactococcus* concerning intestinal barrier function. The results of this project will lead to the selection of *Lactococcus* potential probiotic strain with positive effects on modulation of the function and structure of the gut barrier, as well as the ability to counteract inflammation-induced intestinal barrier disruption. This, in turn, could lay the groundwork for incorporating the selected strain into prophylactic and supportive treatments for inflammatory bowel disease and gut-inflammation-related extraintestinal disorders.