Impact of *Lactococcus* and *Leuconostoc* bacteria on reinforcing gut barrier – from identification of potentially probiotic strains and molecular factors involved in muco-affinity to evaluation of their mucus protective function *in vivo* [description for the general public]

Reasons for attempting a particular research topic: Can we feel safe being inhabited by trillions of microbes? Human gastrointestinal tract is colonized by dense and complex microbial communities, termed gut microbiota, that significantly influence normal physiology and disease susceptibility of the host. Gut barrier plays a crucial role in spatially compartmentalizing bacteria to the lumen. This is achieved through the production of a secreted mucus that limits penetration of bacteria and is fortified by the production of antimicrobial peptides and proteins that kill or inhibit growth of bacteria in proximity to the epithelium. A consequence of perturbations in gut barrier function, for example due to poor nutrition, infection, or other illness, can lead to increased intestinal permeability, also known as the “leaky gut”. Disruption of the gut barrier has been associated with many gastrointestinal diseases, such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), celiac disease, and the early stages of colon cancer development, but also with extra-intestinal pathological conditions, such as diabetes, food allergy or autism spectrum disorders. Considering the strong influence of gut microbiota in the modulation of the function and structure of gut barrier, probiotic treatment is a promising new weapon for reducing intestinal permeability. Despite the fact that lactic acid bacteria (LAB) represent a minor proportion of the bacterial community in the gastrointestinal tract (GIT), their role within a human intestinal microbiota due to interactions of some LAB with the human intestine and their associated health benefits cannot be underestimated. The most studied LAB in terms of the probiotic activities are lactobacilli. However, little is known about host-bacterial interactions of lactococci and leuconostocs, widely used in dairy industry as starter cultures. Notably, there is an increasing evidence that foodborne bacteria are biologically active in the colon and therefore might contribute to the functions of gut microbiota. Mucophylic properties (i.e. ability to attach to mucosal surface, degradation of mucin sugars) can potentially prolong the contact between bacteria and the host and therefore enhance the desired pro-health effect and promote the immune tolerance to commensals and food-borne bacteria, and the immune response to pathogens.

The project goal: The aim of our study is to investigate the impact of the most promising potential probiotic among *Lactococcus* and *Leuconostoc* bacterial strains on reinforcing gut barrier.

Description of research: The selection of *Lactococcus* and *Leuconostoc* bacterial strains, isolated from raw milk and fermented products, will be based on production of probiotic effector molecules such as lactate, short-chain fatty acids (SCFAs), and vitamins, as well as mucophylic properties, relatively poorly understood at the molecular level in other LAB than lactobacilli, and important for probiotic applications. Ten strains showing the best probiotic properties will be selected for the whole genome-sequencing (WGS) and comparative genome analysis. Based on bioinformatic analysis, putative chromosomal and plasmid-encoded molecular factors involved in muco-affinity will be predicted. In order to confirm the role of selected molecular factors in bacterial adhesion, surface proteome analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique will be performed. Plasmid-cured derivatives or deletion mutants will be obtained and analysed on microtiter plates with various coatings (mucin, fibronectin, collagen IV) and in respect to mucin sugars degradation as well as on mucus-secreting epithelial cell line HT29-MTX-E12. Finally, the ability of the selected strain to counteract colonic mucus barrier impairment and its effect on host microbiota will be verified using a mouse model mimicking IBS physiopathology.

Substantial results expected: We expect that the obtained results will add substantial knowledge on the mechanism of muco-affinity and production of specific bacterial end products by various lactococcal and leuconostoc strains and lead to selection of the novel potentially probiotic strain that have a positive effect on modulation of the function and structure of the gut barrier as well as on host microbiota.