Immunomodulatory properties of *B. longum* spp. *longum* CCM 7952 in the treatment of allergic diseases

Bacteria of the genus *Bifidobacterium* belong to a group of microorganisms referred to as pro-health bacteria for the human body. Their presence in the digestive tract of a newborn ensures proper immune system maturation. In the 21st century, the widespread use of antibiotics and a highly-processed diet, especially in developed countries, contributed to a decrease in the number and diversity of bacterial strains, e.g. of the genus *Bifidobacterium* in children (Olm et al., 2022). Disturbances in the qualitative and quantitative composition of the bacterial microflora lead to an increased likelihood of autoimmune or metabolic disorders. The most commonly observed diseases include allergies, chronic sinusitis, inflammatory bowel disease, obesity, and diabetes (Milani et al., 2017b). **According to the World Allergy Organization (WAO), the number of people suffering from allergic diseases already reaches 40% of the world's population (Agache et al., 2019).** It is therefore important to conduct research on new methods of treating allergic diseases. One of the solutions is to increase the diversity of microflora that ensure proper immune responses. The characterization of new bacterial strains together with the assessment of their immunomodulatory properties and methods of their administration will enable the development of more personalized therapies and reduce the occurrence of side effects or the development of bacteremia in immunocompromised patients.

This doctoral thesis presents the characteristics of immunomodulatory properties of selected strains of the genus *Bifidobacterium* tested as live and thermally inactivated bacteria. In order to accurately characterize the tested strains, a wide range of cell lines and a mouse model of sensitization to ovalbumin (OVA) were used. The tested strains differed in the immunomodulatory potential and the degree of absorption and transfer between epithelial cells and dendritic cells. In addition, thermal inactivation changed the cytokine response induced by these strains. Based on the results of stimulation of the respiratory epithelial lines, 4 *Bifidobacterium* strains were selected for further research: Bin 369, Bl 7952, Bad 373, and Ban 218. In the next step, mouse spleen cells sensitized to OVA were stimulated with selected strains. The conducted experiments showed that all tested strains, both live and thermally inactivated bacteria, reduce the level of Th2 pathway cytokines induced by OVA administration. However the strain ***Bifidobacterium longum* ssp. *longum* CCM 7952** (Bl 7952) was the only one of the tested strains that did not induce the production of IFN-γ, a mediator of the Th1 lymphocyte response. Lack of activation of the Th1 pathway, and inhibition of the production of Th2 cytokines indicates that the potential anti-allergic effect of Bl 7952 is based on the activation of other immunomodulatory mechanisms. These properties were checked in the next stage of research using a mouse model of sensitization to OVA. Intranasal administration of live Bl 7952 bacteria leads to a reduction of allergic inflammation both at the local (lung) and systemic levels. Both, the level of Th2 cytokine and specific anti-OVA IgE antibody was decreased. In the lungs, a decrease in the number of immune cells, in particular eosinophils, was observed. The effect obtained after administration of the

thermally inactivated Bl 7952 strain to mice is much weaker. Only a decrease in the level of IL-4 and the number of eosinophils was observed. The simultaneous increase in the number of neutrophils and macrophages in the lungs of OVA-sensitized mice was observed.

These results indicate that the viability status of bacteria has a significant impact on their immunomodulatory properties. The high temperature can cause structural changes in various types of compounds present on the bacterial surface such as proteins, peptidoglycan, lipoteichoic acids, or polysaccharides. This indicates the existence of effector molecules that are responsible for the observed properties. In the next stages of the work, Bl 7952 surface antigens: peptidoglycan, teichoic acids, and polysaccharides were isolated, purified, and then the immunomodulatory properties were determined on selected cell lines and *in vitro* studies on naive cells isolated from the bone marrow and splenocytes. It was shown that the molecules with the greatest immunomodulatory properties were cellular polysaccharides and those isolated from mucus. In the case of LTA, no activity was observed, while peptidoglycan (PGN) induced a rather weak immune response.

The results presented in this paper are the basis for further research on the development of antiallergic preparations containing surface antigens. The Bl 7952 strain is also used by a Ph.D. student in research on the development of a rapid screening test to determine the anti-allergic properties of bacteria using human nasal epithelial cells in research financed by the National Science Center under Preludium 17 entitled: “Intranasal administration of probiotics - development of an *in vitro* model of allergic rhinitis and assessment of the potential of using probiotic strains in its prevention and treatment”.