3. Summary

Breathing is one of the most basic physiological processes, that enables organisms to survive. However, the respiratory tract is exposed to millions of stimuli daily, such as allergens, bacteria, fungi, viruses and air pollution. The body's immune cells determine the appropriate response to these factors (1). They maintain the optimal functioning of our body in constantly changing external conditions, maintaining a state of body balance called homeostasis. Disturbing the homeostasis of the respiratory tract can lead to the development of inflammation, such as allergies. The term "allergy" encompasses the three most prevalent allergic diseases: allergic rhinitis (AR), food allergy (FA) and asthma. Hypersensitivity reactions to harmless molecules in the body characterize allergies. According to the World Allergy Organisation (WAO), AR affects about 40% of the world's population, and asthma affects around 334 million people worldwide (6). Both diseases may result from similar triggers, including pollen, house dust mites, moulds, or proteins inhaled from food.

However, the beneficial effects of microorganisms on the human body were first observed at the beginning of the 20th century (12). Probiotics are currently commonly used alongside antibiotic therapy to supplement the intestinal microflora and prevent the overgrowth of pathogenic bacteria, and their beneficial effect on many diseases is indisputable (16). Probiotic bacteria can alleviate the symptoms of seasonal allergies by acting on various signalling pathways and cellular responses that are critical in the development of allergic reactions. The presented doctoral thesis focuses on discovering the antiallergic properties of the *Bifidobacterium adolescentis* CCDM 370 (B.370) strain and the surface antigen responsible for these functions. To determine these properties precisely , the surface antigens of B.370 were isolated: polysaccharides, peptidoglycan and surface proteins. The anti-allergic potential was assessed by measuring the levels of cytokine produced by splenocytes from ovalbumin (OVA)-sensitized mice. Based on these results, surface proteins were selected as the surface antigen with the strongest anti-allergic properties, and the greatest inhibitory effect on T2 pathway cytokine production. The receptors that recognize isolated antigens were characterized, showing that the NOD2 and TLR2 receptors detect peptidoglycan. At the same time, polysaccharides and surface proteins activate the TLR2 receptor.

In the next stage, the properties of B.370 surface proteins were examined in mouse models. The mouse model of allergy induced by OVA showed that B.370 proteins can reduce allergy symptoms compared to sensitised mice by decreasing the number of cells in BALF, including eosinophils. B.370 proteins also alleviated inflammation in lung tissues, determined based on histological sections and reduced the production of IL-5 in lung cells, a cytokine of the T2 pathway. Based on the study of cytokine secretion by splenocytes isolated from mice sensitized to OVA and treated with B.370 proteins, the antiallergic properties of B.370 proteins were also shown, which reduced the amount of produced cytokine IL-13, which is also classified as a cytokine of the T2 pathway.

In germ-free mice, the neutral effect of B.370 proteins on immune system development was demonstrated. In the second mouse model, pathogen-free mice, known as germ-free, the neutral effect of B.370 proteins on developing the mouse immune system was demonstrated. Administrating of B.370 proteins to pathogen-free mice had no effect on the number of cells in the lungs or the histological image of lung sections. In addition, it did not increase the production of pro-inflammatory cytokines and chemokines from lung cells, it only caused an increase in the production of IL-12p70, which induces the Th1 lymphocyte differentiation, and stimulates the secretion of IFN-γ, leading to the modulation of the immune response. The administration of B.370 proteins also turned out to be neutral for splenocytes isolated from mice, in which B.370 proteins caused an increase in cytokines IL-6 and TNF-α, which are key to the correct differentiation of naive T lymphocytes, the transformation of monocytes into macrophages and and the regulation of the immune response. B.370 proteins also increased the levels of chemokines CCL5 and CCL3, which are associated with recruting immune system cells.

To investigate how B.370 proteins inhibit allergies, studies were conducted using the NLRP3 inflammasome activation model in the THP-1 cell line. The NLRP3 inflammasome plays a key role in developing inflammatory respiratory diseases such as allergic rhinitis, allergic asthma, and COPD, and can significantly exacerbate the progression of these diseases. It is a multiprotein complex that activates caspase-1, which activates interleukins IL-1β and IL-18, leading to their release. The results from this experiment showed that B.370 proteins do not inhibit allergy by inactivating the NLRP3 inflammasome and do not affect its activation pathway. Research indicates that the live strain of *Bifidobacterium adolescentis* CCDM 370 can reduce allergies by inhibiting the activation of the NLRP3 inflammasome. Live *B. adolescentis* CCDM 370 bacteria can inhibit the expression of caspase-1, and affect the reduction of IL-1β protein production and secretion. They thus affect key elements of the NLRP3 inflammasome pathway, inhibiting its activation and alleviating allergy symptoms. The results presented in this work confirm the probiotic effect of the *Bifidobacterium adolescentis* CCDM 370 strain and its antiallergic properties. Moreover, they indicate that the surface proteins of B.370 are responsible for some of these properties. These results constitute the basis for further research on developing antiallergic preparations based on surface antigens. However, research on the effect of B.370 surface proteins on the alleviation of allergy symptoms requires additional research, i.e., determining the specific protein responsible for the strain's antiallergic properties and the underlying mechanism of the observed effect.