Doctoral dissertation

Biological activity of *Bifidobacterium animalis* ssp. *animalis* CCDM 218 and *Bifidobacterium adolescentis* CCDM 368 surface antigens in complex interactions with the host organism - prevention/treatment of allergy diseases

MSc Katarzyna Pacyga-Prus

Supervisor

PhD Sabina Górska, Associate Professor at HIIET PAS

Prepared in a Laboratory of Microbiome Immunobiology

at the Institute of Immunology and Experimental Therapy

of the Polish Academy of Sciences

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Abstract

Airway allergy is defined as a disorder manifested by a hypersensitive reaction of an organism to a harmless molecule called an allergen. This disease is one of the most common healthcare issues in developed countries. It affects not only patients' quality of life but also the world's economy through high healthcare costs and lower performance of allergic patients. Alternative treatments are indispensable to overcome the emerging problem of airway allergy since available therapies are insufficient in some patients and cause numerous side effects. Recent studies connected healthy microbiota with lower allergy risk. The importance of *Bifidobacterium* strains' presence in reducing asthma outcomes was described. However, not only life and proliferating bacteria can beneficially impact a host's health. Effector molecules, metabolites produced by bacteria or their compounds, so-called postbiotics, can show the same or enhanced properties of their bacteria of origin. Due to their easy-to-determine structure, these molecules are perfect candidates for detailed functional studies. Moreover, due to their inanimate state, they cannot transfer antibiotic-resistance genes or cause bacteremia, which increases the safety of their use.

In this doctoral dissertation, the biological potential of the effector molecules produced by *Bifidobacterium animalis* ssp. *animalis* CCDM 218 (Ban218) and *Bifidobacterium adolescentis* CCDM 368 (Bad368) was investigated. First, surface antigens were isolated and purified including peptidoglycan (PG, one per strain), lipoteichoic acids (LTA, one per strain), and polysaccharides (PS) (B.PAT, PS 2, PS 3 for Ban218, and BAP1, PS 2, PS 3 for Bad368). Investigation of the antigen's impact on the splenocytes isolated from OVA-induced mice showed the biggest potential of BAP1 to restore the balance between T helper cells type 1 (Th1) and 2 (Th2). For this reason, it was BAP1 that was subjected to further detailed analysis. The investigation showed efficient transfer of the selected PS between epithelial and immune cells. A comprehensive investigation of the BAP1 structure allowed the determination of a unique hexasaccharide repeating unit consisting of rhamnose, glucose, and galactose residues creating a PS of molecular mass approximately 9.99 x 10^6. Next, the impact of BAP1 was tested in germ-free (GF) mice. It turned out that despite the general neutral effect of this PS on the naïve immune system, it was able to decrease allergy-related chemokine (C-C motif) ligand 2 (CCL2) and eotaxin levels and increase *Rorc* expression in the lungs. Intriguingly, intranasal administration of BAP1 to OVA-allergy mice induced both systemic and local responses. It decreased OVA-specific immunoglobulin (Ig) E in sera as well as IL-10, and Th2-related cytokine production in splenocytes. In the lungs, it reduced inflammation by inhibiting eosinophils and macrophage infiltration together with IL-5, IL-13, IL-4, and IL-10 cytokine production by lung cells. Finally, gene expression analysis recalled the results obtained in GF mice regarding BAP1's impact on the *Rorc* expression, however, the result was insignificant.

Also, it confirmed the role of the tested PS in the inhibition of IL-10 cytokine production since it led to a decrease in *Il10* gene expression.

Among Ban218 surface antigens it was B.PAT which appeared to be the most interesting due to the presence of phosphorus substitution. To investigate whether the introduction of certain modifications to a PS may improve its function, in this work, the B.PAT underwent dephosphorylation. Results showed that this treatment changed the spatial structure of B.PAT, and importantly, it increased PS's immunomodulatory functions in bone marrow dendritic cells (BMDCs). In addition, dephosphorylation enhanced the anti-inflammatory properties of B.PAT in the model of interleukin(IL)-1β inflammation.

Overall, the results presented in this doctoral dissertation positively confirm the great potential of BAP1 to alleviate airway allergies. In the future, extensive research on the BAP1 is needed to fully understand its mechanism of action. Furthermore, clinical studies on humans are necessary to validate results obtained in mice models.