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# Obtaining of the recombinant protein Integration host factor of Burkholderia cepacia and investigating its immunogenic properties

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Most bacteria exist in natural ecosystems as members of a well-defined community of microorganisms called biofilms rather than as individual planktonic cells. In biofilms, the bacterial community is surrounded by an extracellular matrix that consists of polysaccharides, proteins and nucleic acids. Matrix provides resistance to different threats (antibacterial drugs, disinfectants) and due to limited diffusion creates microenvironment for coordinated regulation of bacterial community (i.e. expression of virulence factors and metabolic activity). Biofilm formation in the case of pathogens usually associated with a chronic state of infection process.

Cystic fibrosis is a genetic disorder with a defective mucosal clearance which is a premise of chronic bacterial infections associated with biofilm formation in the lungs [1]. *Burkholderia cepacian complex* (Bcc) - a group of pathogens that causes infections among CF patients. Extracellular heterodimeric protein IHF (Integration Host Factor) is one of the factors, participating in the formation of a biofilm‘s matrix and consists of subunits $\alpha$ and $\beta$. IHF binds extracellular DNA and provides its spatial organization [2].

The aim of our study is assessment of IHF as an antigen for vaccine development and as a target for nanobody-based immunotherapy. Two recombinant sequences of Bcc‘s IHF (IHF$\alpha$ and IHF$\beta$) were cloned in expression vector pET29b, extracted and purified by metal-chelate affinity chromatography. Then its DNA-binding activity was confirmed by electrophoretic mobility shift assay. Analysis showed that both homo- and heterodimer recombinant IHF binds with DNA. Moreover, analysis of the puri[U+FB01]ed protein fraction by Mw. Mass spectrometry showed additional form of protein - not only recombinant IHF proteins but also heterodimers of recombinant IHF subunit ($\alpha$ or $\beta$) of *Bcc* with IHF subunit from *E. Coli* - strain used for recombinant protein expression. This phenomenon confirms the conserved structure of IHF and the ability to form inter-species IHF heterodimers.

Immunogenicity of the obtained proteins were tested by immunization of BALB/C mice. Further, we are investigating the protectivity of the immune response after vaccination with recombinant IHF and developing of IHF-specific nanobodies for passive immunization against biofilm-associated infections.

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