Status of CIPKeBiP: Partner
Title of the project: **Inhibition of *Staphylococcus aureus* cell wall remodeling**
Coordinator: **Prof. Dr. Dušan Turk**
ARRS code: J1-8152 (F)

General information on financing
Duration: 1.5.2017 – 30.4.2020

CIPKeBiP Membership

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**Abstract**
Bacterial cells are surrounded by cell wall that gives them shape and structural stability. The cell wall is a giant peptidoglycan network in which polysaccharide chains are cross-linked with peptidyl connections. During cell growth and division, it has to be remodeled. Under stress or starvation, a community of microorganism called biofilm is formed. Biofilm is extracellular polymeric substance generally composed of extracellular polysaccharides and also DNA and proteins. Cells can leave the biofilm colonies and disperse into surroundings, where they can travel and invade new niches. Processes of cell wall remodeling, biofilm formation, and dispersal of bacteria involve enzymes that synthetize and degrade the structure of cell wall components and biofilms. A number of potential proteins involved in cell wall remodeling were assigned from their genetic sequences, however only a few were experimentally studied and characterized, while their role in biofilm formation is essentially not understood.
*S. aureus* is a widespread commensal bacterium in humans and animals. It colonizes the skin and mucous membranes and in most cases does not affect healthy individuals. However, when the bacteria gain access to inner layers of the body through breaches in skin or in membranes, it may pose a serious threat. S. aureus can cope with hostile conditions encountered in the bloodstream of the living host, a scarce supply of certain nutrients, attacks of the immune system and anti-infective therapy. List of infections it causes includes furuncle, impetigo bullosa, surgical wound infection, pyomyositis, botryomycosis, acute or right-sided endocarditis, epidural abscess, toxic shock syndrome and scalded skin syndrome and more. The huge repertoire of different virulence factors and additional supportive gene products that increase its capability to survive within the living host makes *S. aureus* one of most threatening microorganisms regarding hospital and community-acquired infections. One of the features that makes it so resilient is its ability to form biofilm. Biofilm is a community of microorganism that is attached to a surface and plays a significant role in persistence of bacterial infections. Bacteria within biofilms are several orders of magnitude more resilient to antibiotics, compared with planktonic bacteria. The wide-spread use of antibiotics in recent decades resulted in emergence of antibiotic and multiple antibiotic resistant strains, such as methicillin (MRSA) and vancomycin resistant S. aureus (VRSA). Before 1990s MRSA was only known as a healthcare-associated disease, but by now community acquired infections have emerged in

most areas of the world. For most of the known antibiotics, strains resistant to each, have been isolated.
The threat of the spread of even more resistant *S. aureus* strains urges the development of new antibiotics targeting this organism. Peptidoglycan synthesis was a target for treatment of bacterial infections in humans and animals for almost a century and bacteria developed numerous defense mechanisms against it. Since peptidoglycan cell wall is present only in bacterial cells, enzymes which degrade it may be proven as a valid novel drug targets. Taken together, this prompts us to explore the potential of S. aureus GH73 family of enzymes for their potential as drug targets. All of them are located outside the bacteria (on the cell surface) and therefore easily accessible by drugs.They seem to play major role in cell wall degradation.
We aim to gain novel insight and understanding of the mechanisms underlying remodeling of the peptidoglycan cell wall of *S. aureus* and their biofilms. We will produce peptidoglycan hydrolases of *S. aureus* Mu50 strain in *E. coli*. We will characterize their activity by enzymatic assays, determine their crystal structures and use comparative structure analysis of the closely related proteins for identification of their common features. We will study the role of different amino acid residues in catalysis and substrate recognition will be evaluated by studying their mutants. By exploitation of virtual screening methods, we will design potential inhibitors and ligands, synthesize them, and evaluate their potential in *in vitro* and *in vivo* in bacterial culture assays. Whenever possible, the crystal structure complexes will help us to evaluate the predictions. We will gain insight into the enzymatic mechanism by using in silico approach combining structural and biochemical data. We will evaluate their role in cell proliferation and biofilm formation.

**Phase of the project and its realization**
The first three phases of the project are mostly completed and the final two are in progress.

**Bibliografical references**
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