Status of CIPKeBiP: Coordinator
Title of the project: **Structural insight into the mechanism of Clostridium difficile surface formation**

Coordinator: **Prof.dr. Dušan Turk**
ARRS code: J1-1709 (F)

General information on financing

Duration: 1.7.2019—30.6.2022
Range of financing: 0,53 FTE/year

Participating research organizations

ARRS code	Research organization	Status
2990	Centre of excellence for integrated approaches in chemistry and biology of proteins, Ljubljana	Private research institution (coordinator)
0106	"Jožef Stefan" Institute	Public research institution
0104	National Institute of Chemistry	Public research institution
0787	University of Ljubljana, Faculty of Pharmacy	Public research institution

Membership of the project team

Name	ARRS code	Research area	Position
Guzelj Samo	52379	Pharmacy	Researcher
Dr. Lindič Nataša	31952	Biochemistry and molecular biology	Researcher
Dr. Minovski Nikola	29497	Pharmacy	Researcher
Dr. Mravljak Janez	23419	Pharmacy	Researcher
Dr. Novič Marjana	09775	Chemistry	Researcher
Dr. Pajk Stane	28861	Pharmacy	Researcher
Dr. Perdih Andrej	25493	Pharmacy	Researcher
Dr. Turk Dušan	04988	Biochemistry and molecular biology	Coordinator
Dr. Usenik Aleksandra	26515	Biochemistry and molecular biology	Researcher

Abstract

The cell wall of Gram-positive bacteria is composed of proteins and peptidoglycan with the protruding secondary polysaccharides, which differ in the chemical composition and structure among various species. The cell wall secondary polysaccharides are covalently attached to the peptidoglycan and can account for more than a half of the total cell wall mass. They form a dense network of negative charges and impact cation homeostasis, membrane permeability, antibiotic susceptibility and survival in the host. In many species they also act as the anchors to which the outermost paracrystalline protein surface layer, called the S-layer, is non-covalently attached. We have already begun to elucidate the S-layer structure of *Clostridium difficile*. However, the mechanisms responsible for the organization of the S-layer structure and its attachment to the peptidoglycan remain poorly understood.

C. difficile is a dangerous nosocomial pathogen. When the normal gut microbiota of a patient is compromised, *C. difficile* spores germinate and the infection, even when treated, can reoccur and lead to life-threatening complications. The wide-spread use of broad-spectrum antibiotics in treatment of *C. difficile* infections has already resulted in a number of (multiple) antibiotic resistant strains. Considering that the contact with the host takes place at the surface of the bacterium and that the S-layer of *C. difficile* is an essential virulence factor, it is worthwhile to study the associated processes.
Gene manipulation studies of enzymes involved in biosynthesis of *C. difficile* secondary polysaccharides showed that their impairment leads to bacterial growth defects, diffused cell wall, defective anchoring, altered shedding and deposition of secondary polysaccharides, as well as defects in morphology and assembly of the S-layer, changes in biofilm formation and finally, changes in virulence. This prompts us to study the mechanisms of *C. difficile* S-layer assembly and in particular address the enzymes involved in the biosynthesis of secondary polysaccharides. Moreover, characterization of the respective enzymes might expand our understanding in the biogenesis of other

bacterial cell wall polymers.
With this proposal we plan to gain novel insight into the mechanisms underlying the biosynthesis of secondary polymers and their role in the S-layer assembly. We will study three groups of enzymes involved in biosynthesis of secondary polymers (enzymes involved in mannose conversion, glycosyltransferases, and enzymes attaching the secondary polysaccharides to the peptidoglycan). We will use complementary approaches of molecular biology, crystal structure analysis, enzyme activity measurements, structure-based virtual screening of ligands and classical inhibitor synthesis, biochemical and mass spectroscopy analysis, microscopic (optical, electronic and CryoEM) observations of bacteria or their fragments originating from wild type bacteria and those impaired by gene manipulations and inhibitors.

We believe that research systematically addressing the whole group of the respective enzymes has the potential to deliver fundamental discoveries about their roles in the biosynthesis of secondary polymers and S-layer assembly. Hence, a successful outcome of this project will lay foundations for novel drug discovery programs that have the potential to improve human healthcare in life-threatening situations of *C. difficile* infections in a species-specific manner and thereby reduce risks of widely spreading antibiotic resistance.

Phase of the project and its realization

The project has just started and we are starting with the first phase of the work.

Bibliographical references

Publications will be prepared in the later stages of the project when sufficient experimental data will be obtained.