

Exploring Bacterial OMV (Outer Membrane Vesicles)-sRNAs Mediated Interkingdom Communication with Plants and Fungi (ExplOMV)

Science Fund of the Republic of Serbia, Program IDEAS, 7744906, 2021-2024

Principal Investigator: Dr Dragana Nikolic, IMGGE

Participants from IMGGE: Dr Jelena Samardzic, Dr Bojana Banovic Djeri, Dr Dragana Bosnic, Sofija Nestic

The role of extracellular small RNA (sRNA) in interkingdom communication between microorganisms and their eukaryotic hosts has been revealed recently. Extracellular vesicles (EVs) are a significant delivery route of sRNAs, thus regulating gene expression in the recipient cell. Such interactions have been shown between fungi and plants as well as bacteria and animals but, little is known about sRNA-mediated host responses between bacteria and plants or fungi.

This project aims to study the role of sRNAs in the outer membrane vesicles (OMVs) of the plant growth promoting bacteria (PGPB) (of genera *Paraburkholderia*, *Pseudomonas* and *Enterobacter*) in the naturally occurring communication with plant (*Arabidopsis thaliana*) and phytopathogenic fungi (*Botrytis cinerea*). Further, we will explore the ability of engineered OMV-sRNAs to manipulate fungal genes, as a means to suppress fungal diseases of plants.

To monitor the delivery of the OMV cargo into plant and fungi, OMVs and sRNAs will be fluorescently labeled and OMVs' interaction with recipient cells, and RNA delivery, will be monitored by confocal microscopy. sRNA from OMVs and cells of bacteria, grown separately or in the interaction with the plant or fungus, will be sequenced and compared to define the criteria for the selection of sRNAs to be packed into OMVs. To identify sRNAs delivered by OMVs to recipient cells, sRNA-Seq and bioinformatics analysis of plant and fungal cells upon exposure to bacteria will be performed. The ability of OMV-sRNAs to modulate the expression of predicted target genes in the host cells will be tested both for endogenous bacterial and engineered sRNAs, designed to target recipient's gene(s).

The main expected results: the visualization of OMV cargo delivery to recipient cells; revealing the selection criteria for sRNA packaging into OMVs; and evidence of OMV-sRNAs mediated regulation of host genes will be novel, ground-breaking discoveries, impacting many research fields, from agriculture to medicine. The project will pave the way for using engineered OMVs-sRNAs to modulate plant/fungal physiology, encouraging further studies of the application of this process in biocontrol against phytopathogenic fungi, with a potential to its extrapolation to animal and human fungal pathogens.