

Pavel Hrouzek's group

Supervisor: Berness Peter Falcao (falcao@alga.cz)

Title of the project: How do bacteria steal iron: Determining the specificity of siderophore transporters

For how many student/s: 1

Description of the project:

Siderophores are iron binding compounds secreted by microorganisms when their internal iron reserves are exhausted. The starved cells facilitate siderophore-iron complex uptake via specific membrane transporters. Cyanochelins are amphiphilic siderophores synthesized by cyanobacterial strains that have the biosynthetic gene cluster. Genome mining for the cyanochelin biosynthetic gene clusters revealed their widespread occurrence in cyanobacterial phylum. Cyanobacteria, like other gram negative bacteria, import the siderophore iron complex via the TBBDTs present on the outer membrane.

The siderophore team at the Laboratory of Algal Biotechnology elucidates the structure and function of siderophores produced by cyanobacteria. We currently focus on obtaining new siderophore-producing cyanobacterial strains and associated heterotrophic bacteria from environmental samples to investigate the role of siderophore variants in microbial interactions. Bacteria with appropriate transporters - so called TonB-dependent transporters (TBBDTs) - can benefit from siderophore production by others, however it is not clear how the specificity is achieved or how broad is the spectrum of compounds accepted.

In this short training, we will integrate *in silico* and *in vitro* analysis to determine if TonB-dependent transporters (TBBDTs) required for siderophore, in particular cyanochelin B, uptake are present in non-producer strains, and if these transporters enable utilization of cyanochelin by these non-producer strains. On the flip side, we will also determine if cyanochelin producers can uptake other siderophores with the help of a specific TBBDT. In the proposed project, the student will focus on the following objectives and carry out the following techniques:

- A. Bioinformatic Analysis: Identification of TBBDTs in a cyanochelin producer and bacterial strains; structural and functional characterization of the obtained conserved TonB receptors.
- B. Assessing cyanochelin B-mediated iron uptake: This will be done by cross streaking the cyanochelin B producer strain with tentative non producer strains obtained from objective (A). Analysis of siderophore production in the plate by any of the strains by CAS assay, CAS plate assay or LC-MS.
- C. Evaluation of cyanochelin benefits during iron starvation: This will be done by siderophore feeding assays where cyanochelin B will be provided to tentative non-producing strains; and cross-feeding experiments where cyanochelin B-producer strain will be cocultured with a non-producing strain.

Requirements: The student is expected to be comfortable in speaking and writing in English. Previous laboratory experience with microbiology techniques is an advantage not a necessity. I will give an overview about the known literature about TonB receptors and do the initial preparation of the experiments to fit in the time frame. However the student must be comfortable with handling glassware and preparing stock solutions if required. Study degree is not essential, although there is a slight preference for advanced bachelor student.