

Title:

A novel approach to studying bacterial contractile injection system in an environmental context

Authors:

Vasil A. Gaisin, Corina Hadjicharalambous, Izabela Mujakic, Cristian Villena Alemany, Michal Koblížek, Martin Pilhofer

Institution(s):

Institute of Molecular Biology & Biophysics, ETH Zürich, Switzerland. Institute of Microbiology of the Czech Academy of Sciences, Třeboň, Czechia

Abstract (300 words maximum): :

Conventional omics approaches in environmental microbiology often lead to the loss of information about macromolecular organization of the molecules within the cellular context. This can be a significant disadvantage since many proteins are functional only as macromolecular complexes in particular structural forms. A great example is bacterial contractile injection system (CIS), a syringe-like protein complex whose function is the translocation of molecules into a target cell to affect its state, often inducing lysis. Since the function of CIS is tightly linked to its intracellular localization, proteomics cannot confidently predict the function of novel uncharacterized CISs in natura. To overcome this challenge, we have developed a cryo-electron tomography workflow as a technique complementing metagenomics and proteomics. Additionally, we developed an immuno-electron microscopy protocol to identify and quantify CIS particles in environmental samples. Using this approach, we discovered a novel bacterial CIS in thermophilic multicellular Chloroflexota bacteria populating hot spring mats worldwide. We found that this system is similar phylogenetically and structurally to a recently described cytoplasmic CIS, which was found in multicellular *Streptomyces* and has been shown to be involved in cell cycle regulation. Interestingly, using our approaches, we have discovered that the Chloroflexota cells produce higher number of CIS particles in micro-niches they co-occupy with cyanobacteria and therefore saturated with oxygen during daytime. In agreement with this, we observed intracellular CIS particles in Chloroflexota cells grown only under aerobic conditions in axenic culture. Motivated by this discovery, we searched and analyzed similar CISs in extremophilic bacteria from other lineages. Overall, we have gained an understanding that bacterial cytoplasmic CIS is an overlooked cellular feature of the extremophilic bacteria, which is potentially involved in the cell fate control within microbial community.