Comparative analysis of the activity of MDR pumps in *Salmonella enterica* and *Pseudomonas aeruginosa* using methods of potentiometry and fluorescence spectroscopy

Justina Alesiūtė[1], Sigita Pangonytė[2], Valeryia Mikalayeva[2] and **Rimantas Daugelavičius**[1,2]

[1]Department of Biochemistry and Biophysics, Faculty of Natural Sciences, Vilnius University, Vilnius, Lithuania; [2]Department of Biochemistry and Biotechnologies, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania

Multidrug resistence (MDR) pumps are one of the main reasons of antibiotics resistance in *S. enterica* and *P. aeruginosa* cells. We used electrochemical and spectrofluorimetric methods to assay the pump activity in these cells. Accumulation of ethidium (Et^+) by the cells was registered electrochemically using the selective electrode. Simultaneously accumulation of Et^+ was assayed using fluorescence spectroscopy: the increase in fluorescence was observed because of the dye binding to DNA. In parallel potentiometric measurements of the accumulation of tetraphenylphosphonium (TPP^+) ions in the cells were performed. Results of our experiments revealed that Et^+ ions easier than TPP^+ penetrate the OM of *S. enterica* cells. Effects of RND-family MDR pump inhibitor phenylalanyl-arginyl-β-naphtylamide (PAβN) and the outer membrane (OM) permeabilizing compounds EDTA and Polymyxin B were studied. At high concentrations PAβN not only blocks the activity of MDR pumps but also triggers depolarization of the plasma membrane. Starved and permeabilized cells are the most susceptible to the depolarizing activity of PAβN. We demonstrated that the temperature, the intensity of aeration and the composition of medium differently affect the RND-family pump activity in *P. aeruginosa* and *S. enterica*. In absence of nutritives in the media *S. enterica* RND-family pumps effectively extrude indicator compounds but the presence of glucose increases the efficiency of cell envelope barrier to lipophilic compounds. Our results indicated that in formation of the envelope barrier to lipophilic cations contribution of AcrAB-TolC pump is higher than that of LPS layer of the OM. In contrast to *P. aeruginosa* cells, the alternative efflux pumps in *S. enterica* fail to compensate the loss of the major pump AcrAB-TolC. Experiments with *tolC* or *acrB* gene mutants indicated that defects in both the OM and the PM components of AcrAB-TolC pump almost equally contribute to the loss of cell envelope barrier to lipophilic compounds.