RDHK Family, Regulators dedicated to Organohalide Respiration - Sequence Diversity and Functional Prediction

Mathilde Willemin, Christof Holliger, Julien Maillard

EPFL, Laboratory for Environmental Biotechnology (LBE), Lausanne, Switzerland

Organohalide respiration (OHR) is an anaerobic metabolism by which bacteria conserve energy from the use of organohalide molecules as terminal electron acceptors. Because most organohalides of anthropogenic origin are persistent pollutants, the study of bacteria capable of OHR (OHRB) has a strong environmental interest.

OHRB appear in three major phyla (Firmicutes, Proteobacteria and Chloroflexi) and encode at least one reductive dehalogenase (RdhA) in their genomes. RdhA enzymes are the key catalytic subunit in OHR and their corresponding genes are often surrounded by accessory genes organized in *rdh* gene clusters. Among them, *rdhK* is coding for a member of the CRP/FNR superfamily of transcriptional regulators and is dedicated to the regulation of *rdh* genes in Firmicutes. Upon binding of an effector molecule (i.e. organohalide) the regulator recognizes a specific DNA motif in the promotor region of the target genes in order to recruit the RNA polymerase and activate transcription. So far, based on the characterization of only three RdhK proteins, it was assumed that one RdhK regulator senses specific organohalide compounds which are the substrates of the RdhA enzyme encoded in the same *rdh* gene cluster. Therefore, the identification of the binding partners (both effector molecule and target DNA sequence) for each new RdhK regulator can be used as an indirect way to define potential substrates of yet uncharacterized RdhA enzymes.

Firstly, we aimed to study the diversity of RdhK regulators in Firmicutes. In this regard, BLAST search was used to identify CRP/FNR regulators encoded by genes located in *rdhA* direct vicinity in the available genome sequences of *Dehalobacter* spp. and *Desulfitobacterium* spp. This resulted in a list of 96 RdhK sequences that will be used for sequence similarity analysis in order to define RdhK subgroups. Moreover, a tentative identification of signature motifs will be done through the analysis of individual subgroups.

Finally, our RdhK sequence database will be confronted to the available knowledge on organohalide specificity of characterized RdhA enzymes. This should help predicting the range of effectors and substrates for pairs of RdhK regulators and RdhA enzymes, respectively. Such correspondence could possibly lead to a common type of effectors for a given subgroup of RdhK regulators which would have to be verified through biochemical work.