Explore RdhK based regulatory network of organohalide respiration using a hybrid proteins strategy

Mathilde Willemin[#], Christof Holliger and Julien Maillard

Laboratory for Environmental Biotechnology (ENAC-IIE-LBE), Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland
[#] Corresponding author: mathilde.willemin@epfl.ch

Reductive dehalogenase (*rdh*) gene clusters are encoding proteins that enable organohalide respiring bacteria (OHRB) to couple the degradation of halogenated molecules to energy conservation. The transcription of *rdh* gene clusters is regulated by RdhK regulators belonging to the CRP/FNR-family. RdhK6 (previously called CprK1) in *Desulfitobacterium hafniense* was shown to activate the transcription of the chlorophenol *rdh* genes in presence of 3-hydroxy-4-chlorophenylacetate[1,2]. RdhK effector-binding domain binds to organohalides which triggers protein conformational change and allows the interaction with specific DNA motifs (dehalobox, DB) upstream of *rdh* gene clusters[3,4].

The genome of *Dehalobacter restrictus* PER-K23 encodes 24 *rdh* gene clusters, suggesting a great OHR potential. Each cluster has a *rdhK* paralogue in close proximity[5]. The elucidation of the regulation network represents an indirect way to reveal yet unknown substrates for *D. restrictus*. However, the challenge resides in the fact that for each new RdhK, there are a large number of potential organohalides and possible DB sequences, resulting in a high amount of combinations to be tested.

This project aims to develop a strategy involving RdhK hybrid proteins to screen for DB and organohalides individually. The hybrids are composed by one domain (i.e. DNA- or effector-binding domain) of *D. hafniense* RdhK6 and the complementary domain from any RdhK of interest. The proof of concept as well as strategy limitations and alternatives will be discussed.

References

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