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

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Reductive dehalogenase (*rdh*) gene clusters encode for proteins involved in organohalide respiration (OHR), a bacterial process where organohalide compounds are used as terminal electron acceptors. RdhK proteins, members of the CRP/FNR family of transcription regulator, are dedicated to the regulation of *rdh* gene clusters. To date only a few RdhK proteins have been characterized in *Desulfitobacterium hafniense* while many copies of *rdhK* genes have been identified within OHR bacteria genomes[1,2].

RdhK, when bound to a specific organohalide compound, recognizes a palindromic sequence located in the promoter region of *rdh* genes[3]. Generally, the protein activates the cluster of genes responsible for the respiration of the recognized effector. Therefore, the identification of both binding partners may represent an indirect way to identify new substrates for yet undescribed *rdh* gene clusters.

Visualization of the tripartite complex requires the presence of the interdependent effector and dehalobox partners which makes *in vitro* screening not applicable. To circumvent this problem, the design of hybrid RdhK proteins is proposed here. The idea is to allow the decoupling of the effector screening from the screening of the DNA targets. According to RdhK6 structure, two alternative hybrids were designed[4,5]. The corresponding proteins were purified and tested for *in vitro* interactions. A comparison of their interactions specificity and their potential use to fit the objectives will be presented. Moreover, results obtained will be confronted to the described RdhK mechanism[3,5]. Finally, preliminary results on the application of this method for the characterization of new RdhK proteins will be discussed.

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