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Development of new strategies to produce and characterize PceC, a membrane-bound flavoprotein involved in organohalide respiration

Halogenated organic compounds (so-called organohalides) represent one of the major widespread groundwater pollutants. Organohalide respiration (OHR) is a bacterial anaerobic process in which chlorinated compound, *e.g.* tetrachloroethene (PCE), is used as terminal electron acceptor. *Desulfitobacterium* and *Dehalobacter*, paradigmatic organohalide-respiring bacteria (OHRB), harbour the *pceABCT* gene cluster, representing one model system for the study of PCE respiration. To date, the function of PceA, the key enzyme in the process, and PceT, the dedicated molecular chaperone for PceA maturation, are well defined. However, the role of PceB and PceC are still not elucidated. Analysis of the sequence of PceC (and other members of the RdhC family) revealed the presence of multiple transmembrane segments, a flavin mononucleotide (FMN) binding motif and two conserved CX_3CP motifs. The experiments conducted so far, permitted to express in *E. coli* the FMN-binding domain (FBD) of PceC. However, FBD expression resulted in the formation of inclusion bodies. After denaturation with urea, a strategy was developed to reconstitute FBD in a soluble form by inserting FMN with the help of Ftp1, a flavin-transferase from *D. hafniense*. Moreover, preliminary experiments with the recombinant FBD protein showed redox activity, indicating that PceC may play a role in electron transfer in the metabolism of organohalide respiration. These results invite to conduct the following investigations: *i*) a thorough analysis of the redox properties of FBD using redox titration and UV-vis spectrophotometry and, *ii*) the development of a dedicated strategy for the heterologous production and characterization of the full-length membrane-bound PceC protein.