Identifying the role of complex I in organohalide respiration of *Firmicutes*

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Organohalide-respiring bacteria (OHRB) are capable to link energy conservation with the use of organohalide compounds as terminal electron acceptors. While obligate OHRB relies only on this process for growing, facultative OHRB are able to use alternative electron acceptors. The enzymes catalyzing the final step of organohalide reduction are known as reductive dehalogenases and have been well characterized. However, the complete picture of the respiratory chain remains to be elucidated. Recent genomic and proteomic data suggested new potential players in the respiratory process, such as the respiratory complex I.

Complex I, also called NADH:ubiquinone oxidoreductase (Nuo) is a well-recognized player in the classical respiratory electron transfer processes both in eukaryotes and prokaryotes. Its main role is to allow the coupling of proton pumping through the membrane with the transfer of two electrons from NADH to quinones. In bacteria, the complex is usually composed by fourteen subunits forming three modules: the NADH oxidase module, the quinone reductase module and the proton-pumping module. The presence of an 11-subunits version in *Firmicutes* OHRB, lacking the three subunits forming the NADH module suggests the use of alternative electron donor for the complex. Moreover, it is still unclear how to link the function of complex I with the enzymes involved in OHR. The goal of this study is therefore to investigate the role of complex I in OHRB, focusing on the *Firmicutes* phylum.

Since OHRB are not genetically tractable, their study involves the use of alternative methodology. Therefore, blue-native PAGE will be use to investigate the complex I. The gel band containing members of the complex I will be cut out and send for MS analysis in order to identify possible partners of the complex. Moreover, pull down assays followed by MS analysis will be carry out with the subunits thought to play the role of entry site for the electrons into complex I. In parallel, comparative proteomics are envisaged to reveal the relative occurrence of complex I in the facultative OHRB *Desulfitobacterium hafniense* cultivated in different conditions (e.g. fermentation, organohalide or other respiration). The overall goal is to address the question whether the presence of the complex correlates with specific growth conditions as a first indication for its role. Preliminary results on complex I identification and characterization will be presented and discussed.