Cloning, expression and characterization of Reductive Dehalogenases from *Desulfitobacterium hafniense* DCB2

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Desulfitobacterium hafniense(DCB-2) is a gram positive, spore forming, nitrogen fixing anaerobe with the ability to reduce metal ions such as Fe(III), U(VI), Tc(VII), Mn(IV), Se(VI), As(V) and Cr(VI). It can also NO_3^- ,S^0 S^{2-} , $S_2O_3^{2-}$,CO_2, SO_3^{2-}, citrate, fumarate and humates, reductively dehalogenate chloroorganic compounds like 3,4-dichlorophenol, 3-hydroxylbenzoic acid, tetrachloroethene, trichlorothene. The ability of DCB-2 to carry out both metal reduction and dehalogenation is being targeted by the United States Department of Energy in order to close some of its contaminated sites. Seven ORFs of the DBC-2 genome are similar to cprA(reductive dehalogenase or RDase) gene of D. dehalogenans. Genes induced with 3-chloro-4-hydroxybenzoate (3C4HBA), 3,5-dichlorophenol (DCP), or *ortho*-bromophenol (*o*-BP), were identified using Xeotron® microarrays detection. Competitive hybridization of cDNA prepared from cultures grown by pyruvate fermentation and under the three dehalorespiration conditions indicated that three RDase genes (designated as MENN, MFRS, and MSGV) were induced by 3C4HBA, and two genes (MSSA and VKMN) were induced by both DCP and *o*-BP. The cprA gene contain a nucleotide sequence similar to binding motifs for two Fe_4S_4 clusters or for one Fe_4S_4 cluster and one Fe_3S_4 .

The MENN and VKMN cprA genes were cloned in pET 44c+ and pET12a+ respectively. The recombinant plasmid was amplified in Novablue Singles Competent cells, purified and transformed in BL21(DE3)LysS cells. The VKMN and MENN proteins were overexpressed by induction with IPTG. The calculated mass for VKMN and MENN were 61kDa and 50kDa respectively. The redox properties of the purified proteins were studied.