Subcellular localization of the tetrachloroethene reductive dehalogenase in *Desulfitobacterium hafniense* strains

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The Gram-positive *Desulfitobacterium hafniense* strains PCE-S [1] and Y51 [2] are strict anaerobes, that are able to grow with pyruvate and tetrachloroethene (perchloroethylene, PCE) as energy substrates. The PCE reductive dehalogenase (PceA) in both strains is a corrinoid-containing Fe/S-protein that dechlorinates PCE to cis-1,2-dichloroethene (cDCE) via trichloroethene (TCE). The cytoplasmic precursor of the enzyme (prePceA) bears a Tat (twin arginine translocation) signal peptide for protein export across the cytoplasmic membrane. A recent study on the pce gene expression in the Gram-negative *Sulfurospirillum multivorans* showed that PceA was predominantly found in the periplasm when PCE was present [3]. In the absence of PCE the PCE reductive dehalogenase was detected primarily as prePceA in the cytoplasm. Similar studies for *Desulfitobacterium hafniense* strains PCE-S and Y51 are described here.

To elucidate the membrane-associated localization of the PCE dehalogenase in *D. hafniense* strains grown in the presence or absence of PCE, the freeze-fracture immunogold labeling technique was applied. Using the SDS freeze-fracture replica labeling technique (SDS-FRL) [4], chemically unfixed quick-frozen cells are freeze-fractured and replica-attached membrane proteins can be detected by immunogold labeling.

In *D. hafniense* Y51 grown 15 subcultures (~45 generations, 10% inoculum) on pyruvate/PCE-containing medium (Figure 1) the PCE dehalogenase was detected in small clusters on the exoplasmic fracture face (EF) of the cytoplasmic membrane (Figure 1C). Another fraction of the enzyme was found relatively dispersed in the cytoplasm (Figure 1A) and on the protoplasmic fracture face (PF) of the membrane (Figure 1B).

When the cells were grown repeatedly (15 subcultures) in the absence of PCE (Figure 2) the PCE dehalogenase remained in the cytoplasm and was found in membrane-associated compact bodies or aggregates of high electron density (Figure 2A, B). Almost no PCE dehalogenase label was found on the exoplasmic fracture face (EF) of the cell membrane (Figure 2C).

The analysis of *D. hafniense* PCE-S strain under the same experimental conditions yielded similar results (not shown). The data achieved by immuno electron microscopy were supported by subcellular fractionation experiments and subsequent immunological analyses. The localization pattern of the PCE dehalogenase in *D. hafniense* grown in the presence or absence of PCE principally resembled the results reported for *Sulfurospirillum multivorans* [3]. However, in the absence of PCE the prePceA formed protein aggregates (Figure 2A, B) that were never observed in *S. multivorans* and might be a PCE dehalogenase "reservoir".

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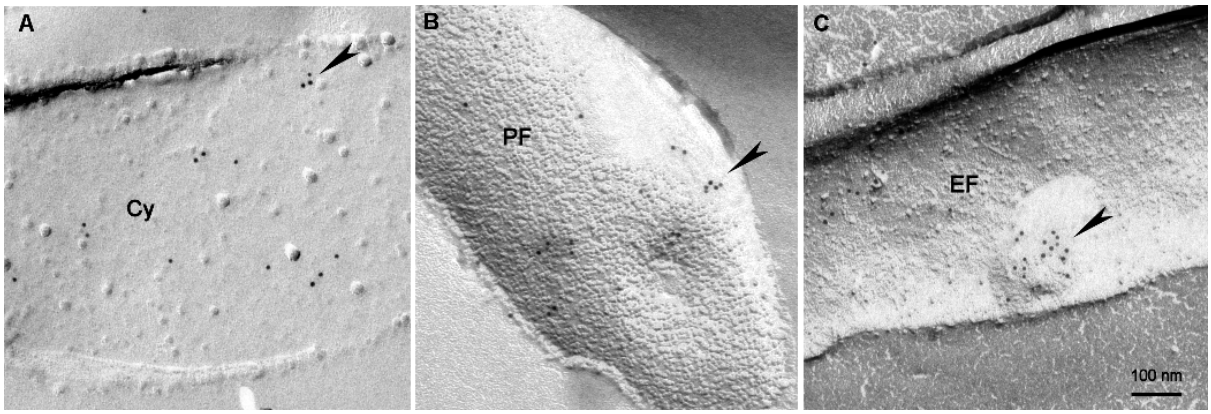

Figure 1. Localization of the PCE dehalogenase by SDS freeze-fracture replica labeling in *Desulfitobacterium hafniense* cells grown for ~45 generations on pyruvate/PCE-conaining medium. The PCE dehalogenase was detected dispersed in the cytoplasm (Cy in **A**), on the protoplasmic fracture face (PF in **B**) of the cytoplasmic membrane and in small patches on the exoplasmic fracture face (EF in **C**) of the cytoplasmic membrane.

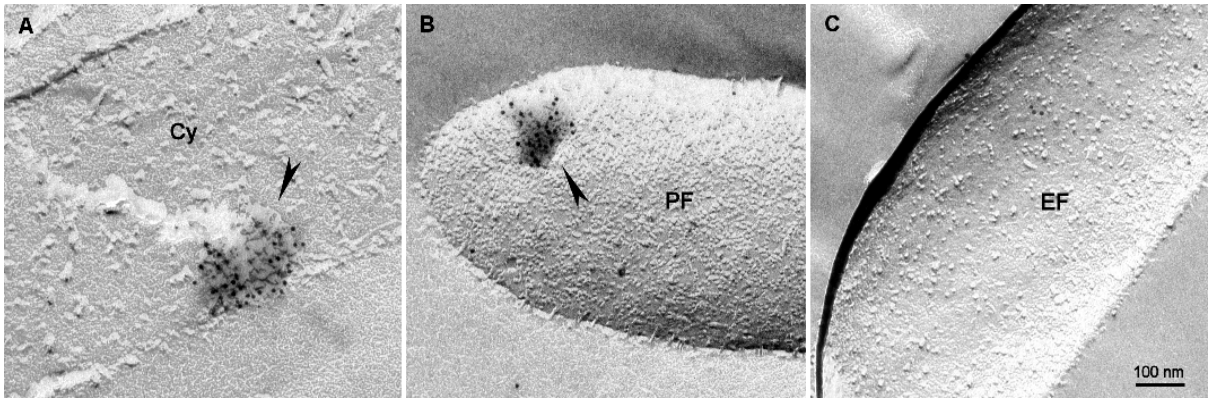

Figure 2. Localization of the PCE dehalogenase by SDS freeze-fracture replica labeling in *Desulfitobacterium hafniense* cells grown for ~45 generations on pyruvate/fumarate-containing medium without PCE. The PCE dehalogenase was detected in the cytoplasm (Cy in **A**) in membrane-associated compact bodies or aggregates of high electron density, also visible on the protoplasmic fracture face (PF in **B**) of the cytoplasmic membrane. Very few PCE dehalogenase indicating label was found on the exoplasmic fracture face (EF) of the cytoplasmic membrane (EF in **C**).

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