Anaerobic biodegradation of organohalide pollutants: a crucial step towards the elucidation of proteins involved

Lorenzo Cimmino[1#], Adrian Schmid[2], Christof Holliger[1] and Julien Maillard[1]

[1] Laboratory for Environmental Biotechnology (ENAC-IIE-LBE), Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

[2] Proteomics Core Facility (PCF), Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

[#] corresponding author: lorenzo.cimmino@epfl.ch

ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE

INTRODUCTION

- Organohalide respiration (OHR) is a bacterial anaerobic process in which halogenated compounds, *e.g.* tetrachloroethene (PCE), are used as terminal electron acceptors (1).
- Our model organisms to study OHR are *Dehalobacter restrictus* (*Dre*-PER-K23) and *Desulfitobacterium hafniense* (i.e. *Dha*-TCE1 and *Dha*-DCB-2) (**Fig.1**).

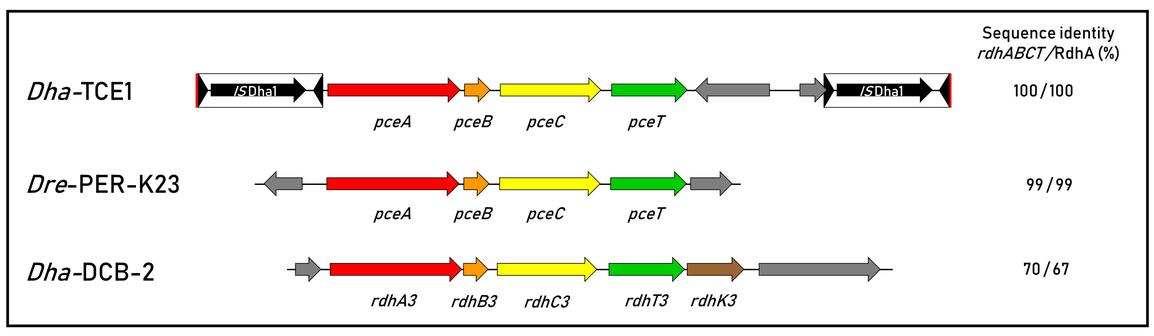

Figure 1. The conserved *rdh*ABCT gene cluster across strains belonging to different OHR genera, taken from (2).

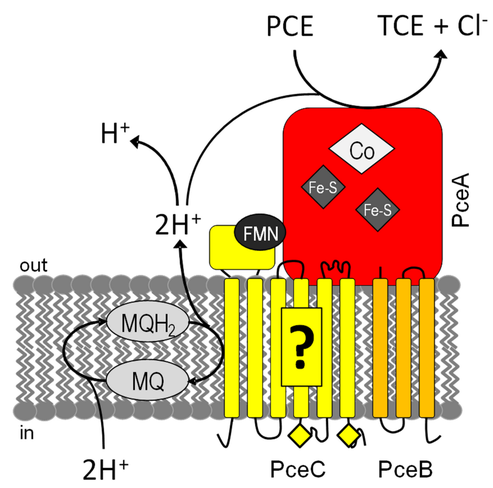

Figure 2. Tentative model of the psossible RDH complex in *D. restrictus*, taken from (3).

- PceA is the well characterized reductive dehalogenase (RdhA) that catalyzes PCE dechlorination, while PceB is the putative membrane anchor for PceA.
- PceC is predicted to be an integral membrane flavoprotein with six transmembrane α-helices, a peripheral FMN domain and two conserved CX_3CP motifs (**Fig.2**) (3).

- The *pceABCT* gene cluster was identified in both *D. restrictus* and *D. hafniense* strain TCE1. The *pce* gene cluster displays 70% sequence identifty with the *rdh-3* gene cluster from strain DCB-2, which serves to dechlorinate 3,5-dichlorophenol (3,5-DCP) (4).

OBJECTIVES

Elucidate the relative stoichiometry of the four subunits (A, B, C and T) of the typical *rdhABCT* gene cluster, in view to understand their possible implication in forming a protein complex in the cytoplasmic membrane. The question is currently addressed both at RNA and protein levels.

RNA level

For the transcriptional analysis of *rdh* genes, *D. hafniense* strain DCB-2 was selected since the *rdh-3* gene cluster is highly similar to the *pce* gene cluster and was shown to be actively regulated by the presence of 3,5-DCP (4), in contrast to *pce* gene clusters in *D. restrictus* and *D. hafniense* strain TCE1 (2).

Transcription analysis of the *rdh-3* genes were performed by reverse transcription (RT)-PCR with RNA from strain DCB-2 spiked with 3,5-DCP (data not shown).

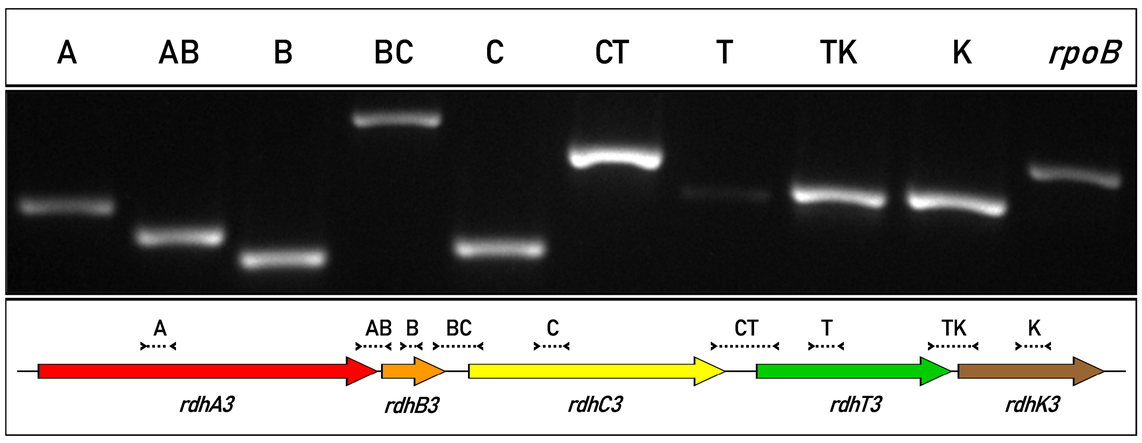

Figure 3. RT-PCR amplification of the *rdh-3* genes from *D. hafniense* strain DCB-2. The top panel shows the results obtained from gel electrophoresis (inc. *rpoB* as housekeeping gene), while the targeted regions of the gene cluster is depicted in the bottom panel.

Evidence was obtained that all individual *rdh* genes were transcribed. More importantly, the data obtained show the co-transcription of all successive gene pairs in the *rdh-3* cluster of strain DCB-2.

This result suggests that the *rdh-3* cluster (and likely other similar *rdh* gene clusters) can be considered as an operon. However, the possibility of additional promoters upstream of individual *rdh* genes within the cluster cannot be excluded.

Protein level

In order to evaluate the relative stoichiometry of A, B, C and T protein subunits, a quantitative proteomics approach is currently under scrutiny on membrane extracts of *D. restrictus*.

All subunits except PceB gave satisfactory peptide coverage using LC-MS/MS with trypsin digestion, offering plethora of possible peptide candidates to be used as references in quantitative proteomic analysis (**Fig.4**).

PceA *(trypsin digestion)*

```
  1 MGEINRRNFLKASMLGAAAAAVASASAVKGMVSPLVADAADIVAPITETSEFPYKVDAKYQRYNSLKNFF
 71 EKTFDPEANKTPIKFHYDDVSKITGKKDTGKDLPTLNAERLGIKGRPATHTETSILFQTQHLGAMLTQRH
141 NETGWTGLDEALNAGAWAVEFDYSGFNAAGGGPGSVIPLYPINPMTNEIANEPVMVPGLYNWDNIDVESV
211 RQQGQQWKFESKEEASKMVKKATRLLGADLVGIAPYDERWTYSTWGRKILKPCKMPNGRTKYLPWDLPKM
281 LSGGGVEVFGHAKFEPDWEKYAGFKPKSVIVFVLEEDYEAIRTSPSVISSATVGKSYSNMAEVAYKIAVF
351 LRKLGYYAAPCGNDTGLSVPMAVQAGLGEAGRNGLLITQKFGPRHRIAKVYTDLELAPDKPRKFGVREFC
421 RLCKKCADACPAQAISHEKDPKVLQPEDCEVAENPYTEKWHLDSNRCGSFWAYNGSPCANCVAVCSWNKV
491 ETWNHDVARIATQIPLLQDAARKFDEWFGYNGPVNPDERLESGYVQNMVKDFWNNPESIKQ
```

PceB *(trypsin digestion)*

```
  1 MNIYDVLIWMALGMTALLIQYGIWRYLKGKGKDTIPLQICGFLANFFFIFALAWGYSSFSEREYQAIGMG
 71 FIFFGGTALIPAIITYRLANHPAKKIRESSDTISA
```

Figure 4. Coverage of detected peptides after trypsin digestion of PceA and PceB. Bold letters highlight all possible cleavage sites for trypsin. Detected peptides are indicated in blue.

Proteomic analysis after chymotrypsin digestion was therefore implemented for PceB, revealing an improved peptide coverage (**Fig.5**).

PceB *(chymotrypsin digestion)*

```
  1 MNIYDVLIWMALGMTALLIQYGIWRYLKGKGKDTIPLQICGFLANFFFIFALAWGYSSFSEREYQAIGMG
 71 FIFFGGTALIPAIITYRLANHPAKKIRESSDTISA
```

Figure 5. Coverage of detected peptides of PceB after chymotrypsin digestion. Bold letters highlight all possible cleavage sites for chymotrypsin. Detected peptides are indicated in grey.

Outlook

- The co-transcription of the gene pairs in the *rdh*-3 gene cluster will be further investigated via quantitative analysis (RT-qPCR). Furthermore, the identification of additional promoters in the intergenic region of individual *rdh* genes will be of main interest to understand the transcriptional regulation of this operon.
- Preliminary data on peptide detection for all four protein subunits will permit to define reference peptides for each of them that will be used for measuring their relative stoichiometry in samples of *D. restrictus* and *D. hafniense* strain TCE1.

References

(1) Schubert *et al.* 2018, *FEMS Microbiol Ecol* **94**:fiy035; (2) Duret *et al.* 2012, *Appl Env Microbiol* **78**, 6121–6127; (3) Buttet *et al.* 2018, *Front Microbiol* **9**:755; (4) Mac Nelly *et al.* 2014, *Appl Env Microbiol* **80**:4313-4322.

Acknowledgements

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