Definition of RdhK subfamily, a CRP/FNR transcription regulator dedicated to Organohalide Respiration

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1. Context

A number of bacteria use halogenated compounds as electron acceptors in an energy-conserving process called organohalide respiration (OHR). Clusters of *rdh* genes encoding for OHR proteins in Firmicutes are often flanked by a gene coding for a CRP/FNR-type transcriptional regulator, known as RdhK[1]. RdhK regulators activate the transcription of its cognate gene cluster in presence of an effector molecule (i.e. organohalides)[2].

Since only few RdhK regulators have been characterized so far[3], the identification of RdhK organohalide effectors represents an indirect way to discover the substrates associated with yet unknown *rdh* clusters. In this work, a survey of the diversity of RdhK proteins was performed in genomes of OHR Firmicutes with the intention to identify sequence signatures which will help narrowing down the possible effectors recognized by specific RdhK regulators.

2. Objectives

1. Define RdhK subfmily members among CRP/FNR family
2. Identify sequence motifs that distinguish RdhK from other CRP/FNR
3. Identify sequence motifs defining possible RdhK subgroups

3. Methodology

RdhK6 from *Desulfitobacterium hafniense* strain DCB-2[2] was used as query to identify CRP/FNR proteins in 19 genomes belonging to the genera *Dehalobacter* and *Desulfitobacterium*.

→ 188 sequences (CRP/FNR diversity in OHR Firmicutes) (Fig.1)

Among the, true RdhK sequences were defined as those proteins encoded within 5 kb distance from the closest *rdhA* gene (coding for the catalytic subunit in OHR metabolism) .

→ 97 sequences (RdhK diversity in Firmicutes OHRB) (Fig.2)

4. Results

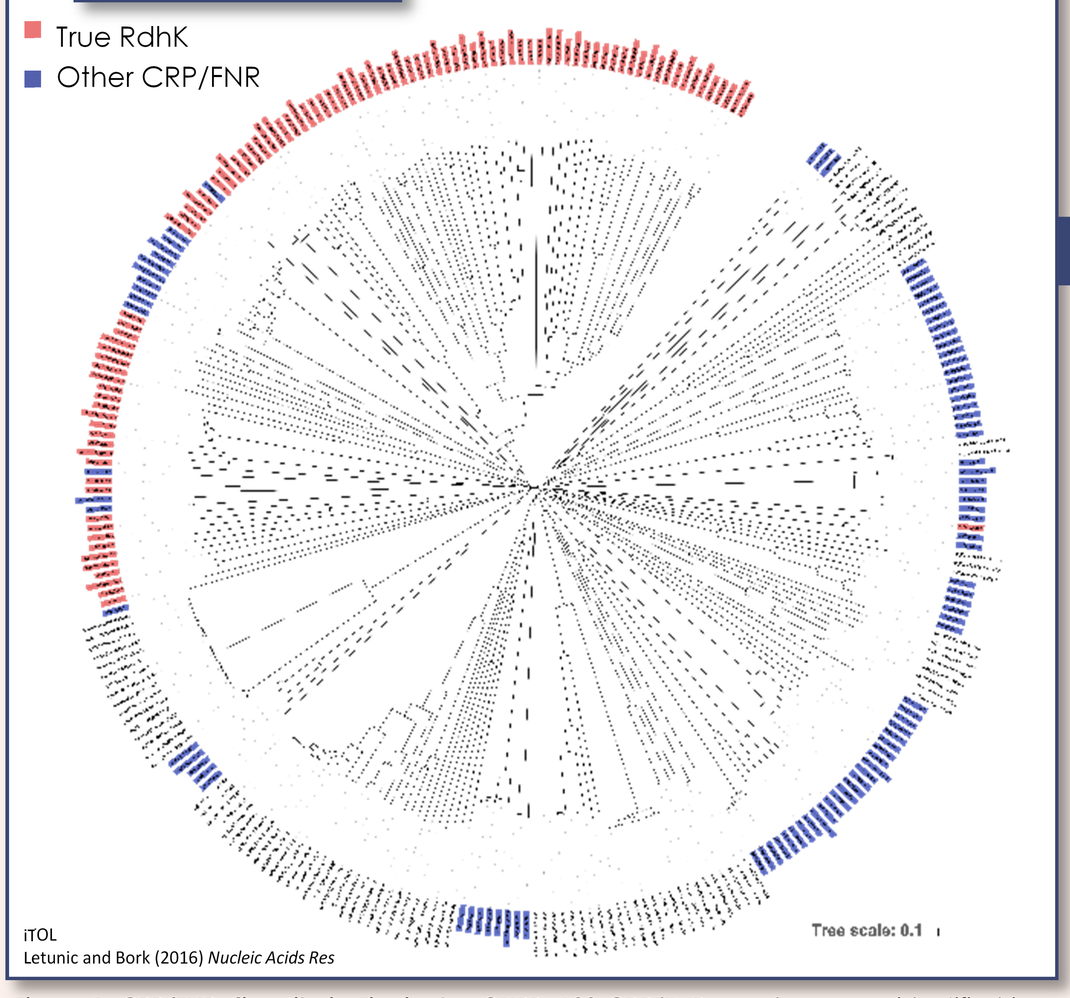


Figure 1. CRP/FNR diversity in Firmicutes OHRB. 188 CRP/FNR members were identified in genomes of *Dehalobacter* and *Desulfitobacterium*, and aligned with CRP/FNR entries from the SwissProt database. True RdhK are indicated in red, while other CRP/FNR from Firmicutes are shown in blue.

Although true RdhK sequences are diverse and rather deeply rooted in the CRP/FNR tree, they form distinct branches, while other CRP/FNR sequences are more scattered.

Only few sequence motifs could be identified in the selection of true RdhK sequences, inviting us to consider them as RdhK signatures:

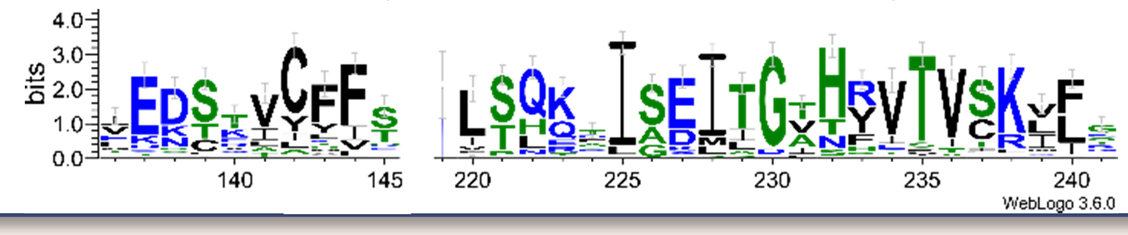
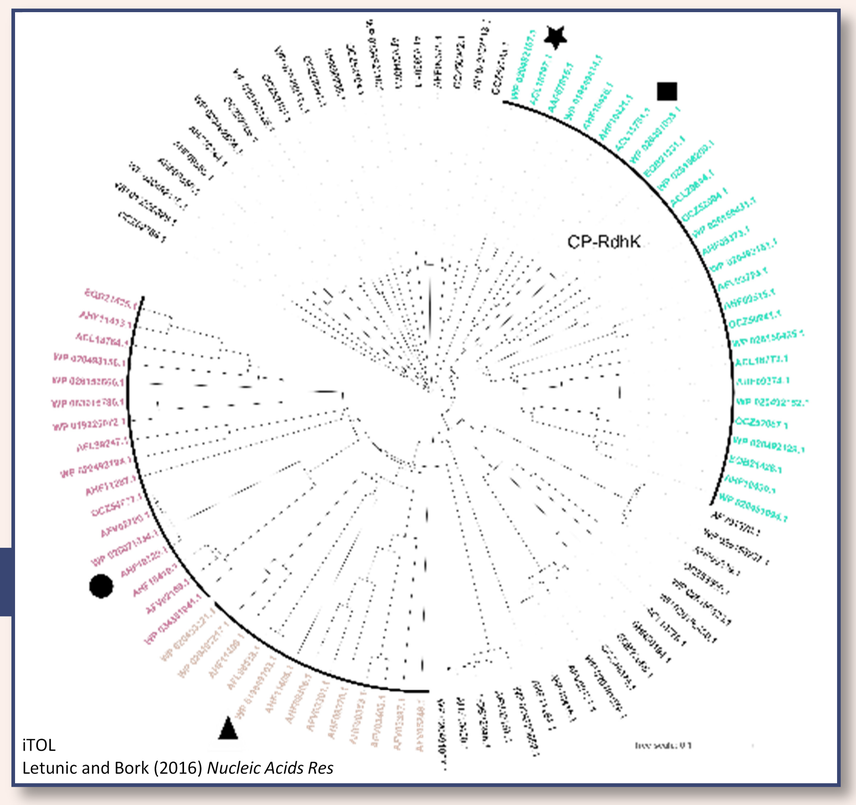


Figure 2. Diversity of 97 true RdhK in OHR Firmicutes. The biochemically characterized chlorophenol (CP) RdhK proteins are indicated as follows: *Dha*-RdhK1 (star) and *Dha*-RdhK6 (square). They belong to a larger RdhK subgroup (in light green). Two RdhK sequences possibly involved in recognizing tetrachloroethene are shown as circle and triangle, and belong to two additional subgroups with a lower conservation degree.



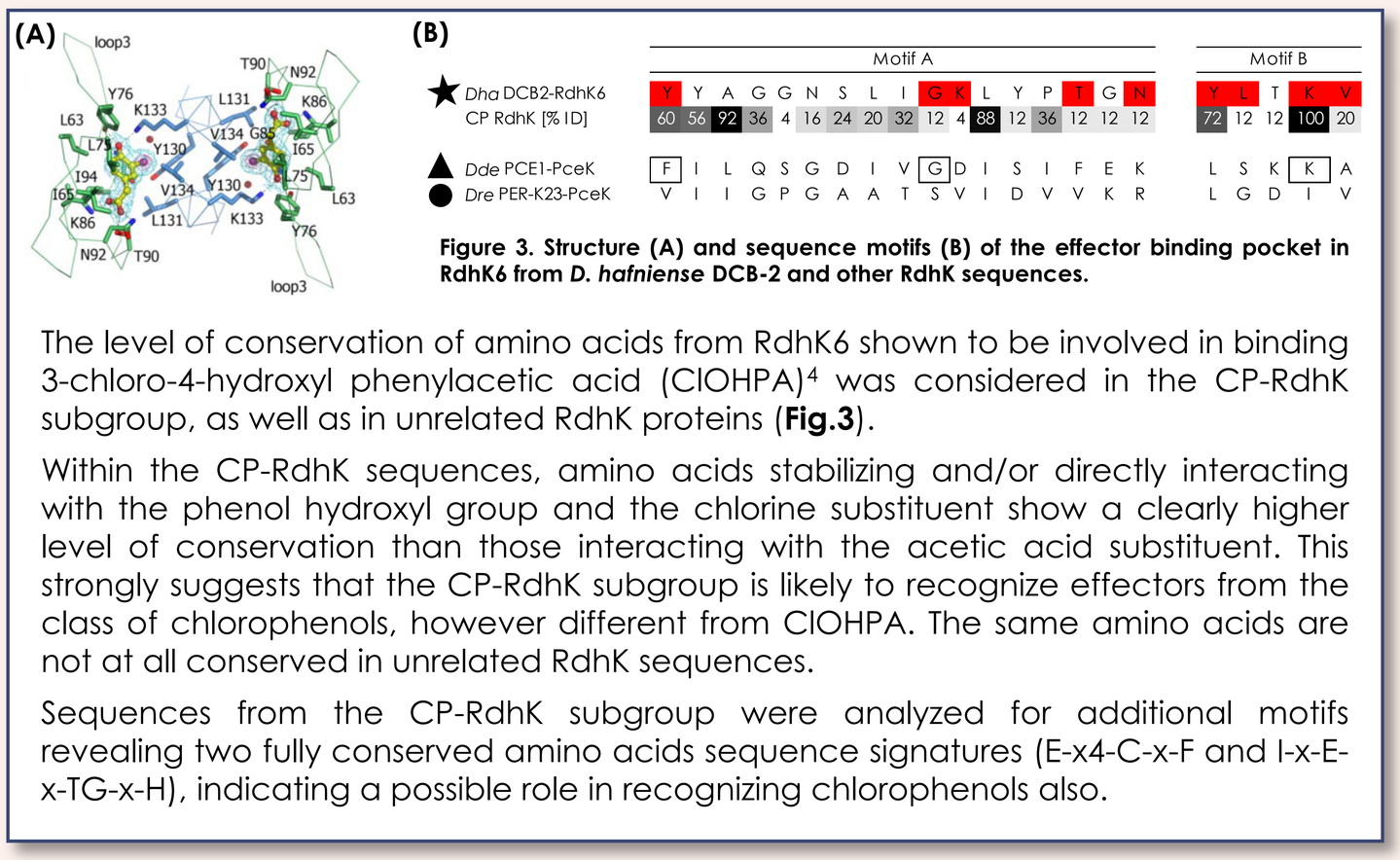


Figure 3. Structure (A) and sequence motifs (B) of the effector binding pocket in RdhK6 from *D. hafniense* DCB-2 and other RdhK sequences.

The level of conservation of amino acids from RdhK6 shown to be involved in binding 3-chloro-4-hydroxyl phenylacetic acid (ClOHPA)[4] was considered in the CP-RdhK subgroup, as well as in unrelated RdhK proteins (**Fig.3**).

Within the CP-RdhK sequences, amino acids stabilizing and/or directly interacting with the phenol hydroxyl group and the chlorine substituent show a clearly higher level of conservation than those interacting with the acetic acid substituent. This strongly suggests that the CP-RdhK subgroup is likely to recognize effectors from the class of chlorophenols, however different from ClOHPA. The same amino acids are not at all conserved in unrelated RdhK sequences.

Sequences from the CP-RdhK subgroup were analyzed for additional motifs revealing two fully conserved amino acids sequence signatures (E-x4-C-x-F and I-x-E-x-TG-x-H), indicating a possible role in recognizing chlorophenols also.

5. Perspectives

- *In vitro* and *in vivo* characterization of new RdhK proteins using sequence features to predict the class of the effector.

→ **Increase screening efficiency**

- Use newly characterized RdhK to develop a model to help in the identification of the effector based on the sequence information.
- Ultimately, use the model to design new RdhK proteins through directed mutagenesis.

→ **Develop tunable organohalide biosensors**

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References

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Abbreviations

CRP = Catabolic repressor protein; FNR = fumarate nitrate regulator; ClOHPA = 3-chloro-4-hydroxyl phenylacetic acid; PCE = tetrachloroethene

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