Perspective

Discovered by genomics: putative reductive dehalogenases with N-terminus transmembrane helixes

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One sentence summary: Recent genomic analysis revealed putative reductive dehalogenase genes from extreme subsurface environments that unlike known reductive dehalogenases have membrane integral domains.

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ABSTRACT

Attempts for bioremediation of toxic organohalogens resulted in the identification of organohalide-respiring bacteria harbouring reductive dehalogenases (RDases) enzymes. RDases consist of the catalytic subunit (RdhA, encoded by rdhA) that does not have membrane-integral domains, and a small putative membrane anchor (RdhB, encoded by rdhB) that (presumably) locates the A subunit to the outside of the cytoplasmic membrane. Recent genomic studies identified a putative rdh gene in an uncultured deltaproteobacterial genome that was not accompanied by an rdhB gene, but contained transmembrane helixes in N-terminus. Therefore, rather than having a separate membrane anchor protein, this putative RDase is likely a hybrid of RdhA and RdhB, and directly connected to the membrane with transmembrane helixes. However, functionality of the hybrid putative RDase remains unknown. Further analysis showed that the hybrid putative rdh genes are present in the genomes of pure cultures and uncultured members of Bacteriodetes and Deltaproteobacteria, but also in the genomes of the candidate divisions. The encoded hybrid putative RDases have cytoplasmic or exoplasmic C-terminus localization, and cluster phylogenetically separately from the existing RDase groups. With increasing availability of (meta)genomes, more diverse and likely novel rdh genes are expected, but questions regarding their functionality and ecological roles remain open.

Keywords: reductive dehalogenase; organohalide respiration; transmembrane helix

INTRODUCTION

With the advent of the Industrial Revolution, human impacts on the environment increased dramatically. Hazardous halogenated organic compounds, organohalogens, were widely distributed in the natural environment through careless use and indiscriminate disposal, and caused major public
concerns due to possible effects on human and environmental health (Häggblom 1992). In attempts for organohalogen bioremediation, a hallmark discovery was the identification of microbes that could use organohalogenes as electron acceptors and reductively dehalogenate them (Sufita et al. 1982). This new metabolism, later termed organohalide respiration (OHR), has found great practical application in bioremediation. Accordingly, bioaugmentation with microbial consortia containing organohalide-respiring bacteria (OHRB) has become a showcase of successful engineered remediation of contaminated environments (Ellis et al. 2000; Stroo, Leeson and Ward 2012).

Over the past three decades, a wealth of knowledge has been obtained about the ecophysiology, biochemistry and environmental distribution of OHRB (Häggblom and Bossert 2003; Adrian and Löffler 2016). Using biochemical, PCR-based and (meta)genomic analysis, reductive dehalogenases (RDases) have been identified as the key enzymes of OHR (Lu et al. 2015; Hug 2016). The RDase-encoding genes (rh) have a conserved operon structure that consists of rhA, coding for the catalytic subunit (RdhA); rhB, coding for a small putative membrane anchor (RdhB) that (presumably) locates the A subunit to the outside of the cytoplasmic membrane; and a variable set of accessory genes (e.g. rhC/TK2ZED) (Kruse, Smidt and Lechner 2016). The catalytic subunits (RdhAa) are characterized by two iron-sulfur clusters (FeS1: CXXCXXCXXCF; FeS2: CXXCXXXCF) and an N-terminal twin-arginine translocation motif (TAT: RRXFXK) (Hollier, Wohlfarth and Diekert 1998). This signal peptide is necessary for secretion of the mature RdhA protein through the cell membrane to the outer side of the cytoplasmic membrane (Smidt and de Vos 2004).

A second type of rhA genes were discovered that lacked TAT motif, were located in the cytoplasm, and lacked respiratory function. This group was termed as ‘catabolic’ reductive dehalogenase that are used to convert organohalogenes to non-halogenated compounds to be used as carbon sources (Chen et al. 2013; Payne et al. 2015). These types of rhA genes were mostly found in marine than terrestrial environments (Reviewed in Atashgahi, Häggblom and Smidt 2018a).

Putative rh genes with N-terminus transmembrane helixes

A recent single-cell genomic study from marine sediments in the Aarhus Bay discovered a third type of potential RDases in uncultured Desulfatiglans-related deltaproteobacterium (Jochum et al. 2018). A single-cell genome (SAG2) contained a putative rh gene that is not accompanied by an rhB, does not encode a TAT signal peptide, and as a unique feature, encodes three transmembrane helices (TMHs) in the N-terminus. Whereas the known respiratory RDases do not have membrane-integral domains, most RhBs have three TMHs (Fig. 1). For instance, similar to the RhdB of Desulfotobacterium hafniense Y51 (Fig. 1A), the putative RDase from the uncultured Desulfatiglans-related deltaproteobacterium (Fig. 1B) has an exoplasmic N-terminus, followed by three TMHs. The remaining C-terminus contains the two binding motifs for FeS clusters, features of the known RDases. However, as the possible catalytic site, the C-terminus is facing the inner side of the cytoplasmic membrane (Fig. 1B) which is a likely localization in absence of the TAT signal peptide. The short cytoplasmic loop between helix 1 and 2 contains the two conserved glutamic acid residues (EXE motif) (Fig. 1B), proposed to play a role in the RdhA–RdhB interaction (Schubert et al. 2018).

Similar cytoplasmic localization of the C-terminus of the putative RDase may enable such an interaction with this loop. Therefore, rather than having a separate membrane anchor protein, this putative RDase is predicted to act like a hybrid of RhdB and RhdA, and likely directly connected to the membrane with the TMHs.

The study of Jochum et al. further revealed that the hybrid putative rh is similar to the putative rh of two deltaproteobacterial pure cultures, i.e. deltaproteobacterium strain NaphS2 and Desulfosulfatatarculus sandiegensis (jochum et al. 2018). Indeed the putative rh genes of these bacteria are not accompanied by an rhB gene, lack TAT motif and contain three N-terminus TMHs. Similar to the putative RDase of the uncultured Desulfatiglans-related proteobacterium obtained from the Aarhus Bay (Fig. 1B), the putative RDase of the strain NaphS2 (Fig. 1C) has cytoplasmic C-terminus. In contrast, the putative RDase of D. sandiegensis has exoplasmic C-terminus (Fig. 1D), similar to the known RDases. The EXE motif in the loop between helix 1 and 2 is facing exoplasm, enabling potential interactions with the exoplasmic C-terminus (Fig. 1D). The three putative RDase share 46%–58% amino acid identity to each other, but share lower identity to the known RDases, e.g. 26%–29% identity to the TceA of Dehalococcoides mccartyi strain195 (DE700279).

The hybrid putative rh genes are widespread

The sequence of the putative RDase of the uncultured proteobacterium obtained from the Aarhus Bay (Jochum et al. 2018) was used as a query in blastp searches against the NCBI non-redundant protein database in December 2018. The results showed that beyond the three identified proteobacterial hybrid putative rh (Jochum et al. 2018), many other similar genes exist in the genomes of pure cultures as well as metagenome-assembled genomes (MAGs) that have gone unrecognized so far (Table 1). The majority of the sequences have three TMHs (detected using TMHMM Server v. 2.0 (Sonnhammer, Von Heijne and Krogh 1998)), the EXE motifs in their N-terminus, and either cytoplasmic or exoplasmic C-terminus containing the two FeS motifs (Table 1, Fig. 2). The C1–C5 regions from known the RDases are also conserved among the hybrid putative RDases (Fig. S1, Supporting Information), however, they are clustered phylogenetically separately from the existing RDase groups (Hug et al. 2013; Hug 2016). The C1–C5 regions from known the RDases are also conserved among the hybrid putative RDases (Fig. S1, Supporting Information). Notably, the majority of the putative RDases are annotated as hypothetical proteins during automated annotation of the genomes.

Of the 11 pure cultures containing hybrid putative rh in their genomes, eight belong to the Marinilabiliales order within Bacteroidetes, that have been isolated from water or sediment samples in marine environment (Table 1). Among these, three strains belong to the genus Mariniflum, Gram-negative facultative anaerobes that can tolerate moderate salt concentrations (Na et al. 2009; Ruvira et al. 2013; Fu et al. 2018). Interestingly, hybrid putative rh genes were also found in the MAGs of uncultured Marinilabiliales obtained from perchlorate-reducing...
Table 1. List of the hybrid putative RDases with TMHs in their N-terminus. Sequence information and the predicted functions by the automated annotation for each sequence are included in Supporting Information.

| Organism | Length (aa) | TMH | C-terminus orientation | GenBank accession number | Sample source used for (meta)genome sequencing | Reference |
|----------|-------------|-----|------------------------|--------------------------|-----------------------------------------------|-----------|
| Deltaproteobacteria bacterium | 482 | 3 | Cytoplasmic | - a | Marine sediment from Aarhus Bay (Jochum et al. 2018) | (Jochum et al. 2018) |
| Dethiosulfatarculus sandiegensis | 487 | 3 | Exoplasmic WP | 246 4279 | Pure deltaproteobacterial culture isolated from a methanogenic long-chain paraffins degrading consortium obtained from marine sediments (Davidova et al. 2016) | (Davidova et al. 2016) |
| Deltaproteobacterium NaphS2 | 478 | 3 | Cytoplasmic IFK11122 | Pure deltaproteobacterial culture isolated from naphthalene-degrading enrichment obtained from marine sediments (Galushko et al. 1999; Didonato Jr et al. 2010) | (Galushko et al. 1999; Didonato Jr et al. 2010) |
| Marinilabiliales bacterium strain SPP2 | 459 | 3 | Exoplasmic WP | 09 642 9615 | Pure Marinilabiliales culture isolated from the Antarctic marine sediment (Watanabe, Kojima and Fukui 2018) | (Watanabe, Kojima and Fukui 2018) |
| Marinilabiliales bacterium | 456 | 3 | Exoplasmic WP | 05 471 5848 | Pure Marinilabiliales culture isolated from tidal flat sediment in Korea (Na et al. 2009) | (Na et al. 2009) |
| Marinilabiliales bacterium breve | 457 | 3 | Cytoplasmic WP | 110 360 576 | Pure Marinilabiliales culture isolated from the Yongle Blue Hole in the South China Sea (Fu et al. 2018) | (Fu et al. 2018) |
| Marinilabiliales bacterium flexuosum | 454 | 3 | Cytoplasmic WP | 120 240 634 | Pure Marinilabiliales culture isolated from coastal Mediterranean Sea water (Ruvira et al. 2013) | (Ruvira et al. 2013) |
| Ancylomarina sp. M1P | 450 | 3 | Exoplasmic WP | 125 029 802 | Pure Marinilabiliales culture isolated from Black Sea water Unpublished | Unpublished |
| Labilibaculum filiforme | 454 | 3 | Exoplasmic WP | 101 260 201 | Pure Marinilabiliales culture isolated from the subsurface sediments of the Baltic Sea (Vandieken et al. 2018) | (Vandieken et al. 2018) |
| Labilibacter marinus | 444 | 3 | Cytoplasmic WP | 06 663 2432 | Pure Marinilabiliales culture isolated from marine sediment at Weihai in China (Li et al. 2015; Lu et al. 2017) | (Li et al. 2015; Lu et al. 2017) |
| Salinivirga cyanobacteriivorans | 453 | 3 | Cytoplasmic WP | 05 795 4221 | Pure Marinilabiliales culture isolated from the suboxic zone of a hypersaline cyanobacterial mat (Ben Hania et al. 2017) | (Ben Hania et al. 2017) |
| Caldithrix abyssi | 444 | 3 | Cytoplasmic WP | 069 30498 | Pure Caldithrixial culture isolated from Mid-Atlantic Ridge hydrothermal vent | (Mironshichenko et al. 2003; Kudinov et al. 2017) |
| Deltaproteobacteria bacterium | 491 | 3 | Exoplasmic | RLB29679 | Hydrothermal sediments (Dombrowski, Teske and Baker 2018) | (Dombrowski, Teske and Baker 2018) |
| Deltaproteobacteria bacterium | 451 | 3 | Exoplasmic | RLB34449 | Hydrothermal sediments (Dombrowski, Teske and Baker 2018) | (Dombrowski, Teske and Baker 2018) |
| Deltaproteobacteria bacterium | 455 | 3 | Exoplasmic | RCO6278 | Hydrothermal sediments (Dombrowski, Teske and Baker 2018) | (Dombrowski, Teske and Baker 2018) |
| Deltaproteobacteria bacterium | 456 | 3 | Exoplasmic | RLB0792 | Hydrothermal sediments (Dombrowski, Teske and Baker 2018) | (Dombrowski, Teske and Baker 2018) |
| Deltaproteobacteria bacterium | 455 | 3 | Exoplasmic | RLC22838 | Hydrothermal sediments (Dombrowski, Teske and Baker 2018) | (Dombrowski, Teske and Baker 2018) |
| Deltaproteobacteria bacterium | 414 | 3 | Exoplasmic | RLC21098 | Hydrothermal sediments (Dombrowski, Teske and Baker 2018) | (Dombrowski, Teske and Baker 2018) |
| Deltaproteobacteria bacterium | 497 | 3 | Exoplasmic | RLB22016 | Hydrothermal sediments (Dombrowski, Teske and Baker 2018) | (Dombrowski, Teske and Baker 2018) |
| Organism | Length (aa) | TMH | C-terminus orientation | GenBank accession number | Sample source used for (meta)genome sequencing | Reference |
|----------|-------------|-----|------------------------|--------------------------|-----------------------------------------------|-----------|
| Delta-proteobacteria bacterium | 359 | 3 | Cytoplasmic | JQZ502398 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Desulfobacteraceae bacterium | 4572 | 187 | Exoplasmic | OQY12990 | Hydrothermal sediment | (Dombrowski et al. 2017) |
| Desulfobacteraceae bacterium | 4572 | 89 | Exoplasmic | OQY53460 | Hydrothermal sediments | (Dombrowski et al. 2017) |
| Bacteroidetes bacterium | 457 | 3 | Exoplasmic | RLD45891 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Bacteroidetes bacterium | 447 | 3 | Exoplasmic | RLD65038 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Bacteroidetes bacterium | 457 | 3 | Exoplasmic | RLD32997 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Bacteroidetes bacterium | 402 | 1 | Exoplasmic | RLD55593 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Bacteroidetes bacterium | 469 | 3 | Cytoplasmic | RLD42118 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Bacteroidetes bacterium | 448 | 249 | Cytoplasmic | OQX80664 | Hydrothermal sediment | (Dombrowski et al. 2017) |
| Bacteroidetes bacterium | 476 | 4 | Cytoplasmic | RLD38167 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Bacteroidetes bacterium | 454 | 3 | Cytoplasmic | RLD75418 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Acidobacteria bacterium | 450 | 3 | Cytoplasmic | RLE20106 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Chloroflexi bacterium | 453 | 3 | Exoplasmic | RLD03862 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Chloroflexi bacterium | 457 | 3 | Exoplasmic | RLD00869 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Chloroflexi bacterium | 453 | 3 | Cytoplasmic | RLD11393 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Bacterium | 457 | 3 | Cytoplasmic | RKZ14043 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Bacterium | 448 | 3 | Cytoplasmic | RKZ19839 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Candidate division KSB1 bacterium | 457 | 3 | Exoplasmic | RKY76399 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Candidate division KSB1 bacterium | 448 | 87 | Exoplasmic | OQX85480 | Hydrothermal sediments | (Dombrowski et al. 2017) |
| Candidate division Zixibacteria bacterium | 501 | 3 | Exoplasmic | RKX26209 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Candidate division Zixibacteria bacterium | 461 | 3 | Cytoplasmic | RKX27199 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Candidatus Aminicenantes bacterium | 448 | 214 | Cytoplasmic | OQX52307 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Candidatus Omnitrophica bacterium | 469 | 3 | Exoplasmic | RKY41132 | Hydrothermal sediments | (Dombrowski, Teske and Baker 2018) |
| Deltaproteobacteria bacterium | 463 | 3 | Exoplasmic | PLX41189 | Perchlorate-reducing communities | (Barnum et al. 2018) |
| Salinivirgaceae bacterium | 497 | 4 | Exoplasmic | PLX17815 | Perchlorate-reducing communities | (Barnum et al. 2018) |
| Marinilabiliales bacterium | 456 | 3 | Exoplasmic | PLW95329 | Perchlorate-reducing communities | (Barnum et al. 2018) |
| Marinilabiliales bacterium | 446 | 3 | Exoplasmic | PLW99613 | Perchlorate-reducing communities | (Barnum et al. 2018) |
| Marinilabiliales bacterium | 455 | 3 | Cytoplasmic | PLW92978 | Perchlorate-reducing communities | (Barnum et al. 2018) |
| Marinilabiliales bacterium | 458 | 3 | Cytoplasmic | PLX09622 | Perchlorate-reducing communities | (Barnum et al. 2018) |
| Marinilabiliales bacterium | 452 | 3 | Cytoplasmic | PLX02242 | Perchlorate-reducing communities | (Barnum et al. 2018) |
| Organism                                      | Length (aa) | TMH | C-terminus orientation | GenBank accession number | Sample source used for (meta)genome sequencing | Reference                                                                 |
|----------------------------------------------|-------------|-----|------------------------|--------------------------|------------------------------------------------|--------------------------------------------------------------------------|
| Bacteroidetes bacterium GWE2, 32, 14         | 432         | 2   | Exoplasmic             | OFX85901                 | Aquifers                                      | (Anantharaman et al. 2016)                                                |
| Bacteroidetes bacterium GWE2, 40, 15          | 462         | 3   | Exoplasmic             | OFX81662                 | Aquifers                                      | (Anantharaman et al. 2016)                                                |
| Candidatus Fischerbacteria bacterium RBG, 13, 37, 8 | 447         | 3   | Cytoplasmic            | OFG65237                 | Aquifers                                      | (Anantharaman et al. 2016)                                                |
| Desulfobacterales bacterium                  | 456         | 3   | Cytoplasmic            | OGR28476                 | Aquifers                                      | (Anantharaman et al. 2016)                                                |
| Desulfo bacteriaceae bacterium                | 476         | 3   | Exoplasmic             | RPI80002                 | Wetlands                                      | (Martins et al. 2018)                                                    |
| Delta proteobacteria bacterium                | 468         | 3   | Exoplasmic             | RJ96807                  | Wetlands                                      | (Martins et al. 2018)                                                    |
| Bacteroidales bacterium                      | 454         | 3   | Exoplasmic             | RPH31952                 | Wetlands                                      | (Martins et al. 2018)                                                    |
| Bacterium SM23, 31                           | 446         | 3   | Cytoplasmic            | KF88368                  | Estuary sediments                            | (Baker et al. 2015)                                                      |
| Candidate division Zixibacteria bacterium SM23, 7, 12 | 441         | 3   | Cytoplasmic            | KU04245                  | Estuary sediments                            | (Baker et al. 2015)                                                      |
| Latescibacteria bacterium DG, 63              | 453         | 3   | Cytoplasmic            | KFY61247                 | Estuary sediments                            | (Baker et al. 2015)                                                      |
| Delta proteobacteria bacterium                | 542         | 3   | Exoplasmic             | PKN64391                 | Deep terrestrial subsurface sediments         | (Hernsdorf et al. 2017)                                                  |
| HGW-Delta proteobacteria-15                   | 459         | 3   | Exoplasmic             | PKX82132                 | Deep terrestrial subsurface sediments         | (Hernsdorf et al. 2017)                                                  |
| Candidate division Zixibacteria bacterium HGW-Zixibacteria-1 | 489         | 3   | Cytoplasmic            | RJK39500                 | Deep terrestrial subsurface fluids            | (Momper et al. 2017)                                                     |
| Marinimicrobia bacterium 46, 43               | 453         | 3   | Exoplasmic             | KUK91590                 | Oil Reservoirs                                | (Hu et al. 2016)                                                         |
| Candidatus Korarchaeota archaeon               | 452         | 3   | Exoplasmic             | PMB78244                 | Hot springs                                   | (Wilkins et al. 2018)                                                    |
| Desulfobacterales bacterium SS, 13, 16 MH16   | 488         | 3   | Exoplasmic             | OEU64881                 | Marine sediments                              | Unpublished                                                              |
| Candidate division KS, 81 bacterium           | 432         | 3   | Cytoplasmic            | RQW00415                 |                                               | Unpublished                                                              |

*Not available; sequence information provided in Supporting Information

b Not available
enrichment cultures originating from marine sediments (Barnum et al. 2018). These genomes mostly lacked respiratory perchlorate, chlorate, oxygen and sulfur reductases and were proposed to be specialized for the fermentation of dead cells (Barnum et al. 2018). These finding indicate an important role of the hybrid putative \( rdh \) genes in Marinilabiliales members. Another pure culture harbouring the hybrid putative \( rdh \) in its genome is Calditrichia abyssi, a thermophilic anaerobic bacterium isolated from a Mid-Atlantic Ridge hydrothermal vent (Miroshnichenko et al. 2003). Calditrichaeota are abundant seabed microbes with genomic potential to degrade detrital proteins through the use of extracellular peptidases (Marshall et al. 2017).

Except the MAGs obtained from the marine perchlorate-reducing enrichment cultures (Barnum et al. 2018), all other MAGs-containing hybrid putative \( rdh \) were obtained from harsh environments such as hydrothermal vents.

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**Figure 1.** Predicted topology of the PceB protein of *D. hafniense* Y51 (A), and N-terminus TMHs of the hybrid putative RDases from uncultured deltaproteobacterium (SAG2) obtained from the Aarhus Bay (B), deltaproteobacterium strain NaphS2 (C), and *D. sandiegensis* (D). The position of the EXE motif is indicated by a star. Note that in panel B, C and D, only partial sequences of the hybrid putative RDases containing N-terminus TMHs were shown. TMHs were detected using TMHMM Server v. 2.0 (Sonnhammer, Von Heijne and Krogh 1998). Permission to reprint panel A was obtained from (Schubert et al. 2018).
Figure 2. Sequence alignment of the hybrid putative RDases. Only conserved sequence motifs among experimentally characterized RDases (TAT, FeS1, FeS2), and the conserved glutamic acid residues (EXE) are included. The accession numbers are ordered according to Table 1, except the first accession number that belongs to TceA of Dehalococcoides mccartyi strain 195. ClustalW (Thompson, Higgins and Gibson 1994) multiple sequence alignment was conducted using BioEdit version 7.2.5 (http://bioedit.software.informer.com/).
Outstanding questions

Genomics and allied technologies have greatly increased the diversity of putative rdh genes in recent years, and extended their distribution from contaminated environments to deep subsurface (Table 1), Antarctic soils (Zlamal et al. 2017), and even human and animal intestinal tract (Atashgahi et al. 2018b). With the expanding availability of the bacterial genomes and increasing application of deep sequencing in diverse environments, much more diverse and likely novel rdh genes are expected in future. This brings forward major open questions:

- Do the newly discovered genes encode RDases? If they indeed encode RDases, what are their functions? Three roles have been shown for the known RDases: energy conservation by OHR, and facilitated fermentation of organic substrates (e.g. pyruvate, lactate or yeast extract) by reoxidation of respiratory cofactors for membrane-bound RDases, and catabolic reductive dehalogenation for cytoplasmic RDases (Fincker and Spormann 2017). Can the hybrid putative RDases with cytoplasmic C-terminus be involved in catabolic reductive dehalogenation, facilitated fermentation or both? In turn, how are the hybrid putative RDases with exoplasmic C-terminus secreted through the cell membrane in absence of TAT signal peptide?
- If indeed involved in reductive dehalogenation, what are the physiological organohalogen substrates of the hybrid putative RDases? The lack of correlation between the rdh sequences and their organohalogen substrates has precluded the ability to predict substrates for novel genes, and to test their functionality using the predicted organohalogen.
- Why the majority of the environmental hybrid putative rdh sequences and rdh-containing pure cultures have been obtained from harsh environments? Can it be that their physiological organohalogen substrates are found in these environments?
- What are the ecological functions of the microbes containing (the hybrid putative) RDases? Detoxification of organohalogs and thereby securing a hospitable environments for themselves and the nearby organisms? Providing carbon sources for themselves (catabolic RDase) or others (respiratory RDase)?

- Can (the hybrid putative) RDases be involved in the production of halogenated bioactive compounds as was shown for biosynthesis of marine bacterial pyrroles mediated by a reductive debranomerase that utilizes a reduct thiol mechanism (El Gamal et al. 2016)? Likewise, can the RDases participate in the production of halogenated bioactive compounds in Eukaryotes such as sponges that are known to harbour Deltaproteobacteria with rdh genes (Wilson et al. 2014; Liu et al. 2017)?

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