Organohalide-respiring *Desulfoluna* species isolated from marine environments

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Abstract

The genus *Desulfoluna* comprises two anaerobic sulfate-reducing strains, *D. spongiiphila* AA1^T and *D. butyratoxydans* MSL71^T, of which only the former was shown to perform organohalide respiration (OHR). Here we isolated a third member of this genus from marine intertidal sediment, designed *D. spongiiphila* strain DBB. All three *Desulfoluna* strains harbour three reductive dehalogenase gene clusters (*rdhABC*) and corrinoid biosynthesis genes in their genomes. Brominated but not chlorinated aromatic compounds were dehalogenated by all three strains. The *Desulfoluna* strains maintained OHR in the presence of 20 mM sulfate or 20 mM sulfide, which often negatively affect OHR. Strain DBB sustained OHR with 2% oxygen in the gas phase, in line with its genetic potential for reactive oxygen species detoxification. Reverse transcription-quantitative PCR (RT-qPCR) revealed differential induction of *rdhA* genes in strain DBB in response to 1,4-dibromobenzene or 2,6-dibromophenol. Proteomic analysis confirmed differential expression of *rdhA1* with 1,4-dibromobenzene, and revealed a possible electron transport chain from lactate dehydrogenases and pyruvate oxidoreductase to RdhA1 via menaquinones and either RdhC, or Fix complex (electron transfer flavoproteins), or Qrc complex (Type-1 cytochrome c3:menaquinone oxidoreductase).

Introduction

More than 5,000 naturally produced organohalides have been identified, some of which have already been present in a variety of environments for millions of years [1]. In particular, marine environments are a rich source of chlorinated, brominated and iodinated organohalides produced by marine algae, seaweeds, sponges, and bacteria [2], Fenton-like [3] and photochemical reactions, as well as volcanic activities [4, 5]. Such a natural and ancient presence of organohalogens in marine environments may have primed development of various microbial dehalogenation metabolisms [6]. Furthermore, marine environments and coastal regions in particular are also commonly reported to be contaminated with organohalogens from anthropogenic sources [7].

During organohalide respiration (OHR) organohalogens are used as electron acceptors, and their reductive dehalogenation is coupled to energy conservation [8-10]. This process is mediated by reductive dehalogenases (RDases), which are membrane-associated, corrinoid-dependent, and oxygen sensitive proteins [9-11]. The corresponding *rdh* gene clusters usually consists of *rdhA* encoding the catalytic subunit, *rdhB* encoding a putative membrane anchor protein [10], and a variable set of accessory genes encoding RdhC and other proteins likely involved in regulation, maturation and/or electron transport [12, 13]. The electron transport chain from electron donors to RDases has been classified into quinone-dependent (that rely on menaquinones as electron shuttles between electron donors and RDases) and quinone-independent pathways [9, 10, 14]. Recent studies suggested that RdhC may serve as electron carrier during OHR in *Firmicutes* [15, 16].

OHR is mediated by organohalide-respiring bacteria (OHRB), which belong to a broad range of phylogenetically distinct bacterial genera. OHRB belonging to *Chloroflexi* and the genus *Dehalobacter* (*Firmicutes*, e.g. *Dehalobacter restrictus*) are specialists restricted to OHR, whereas proteobacterial OHRB and members of the genus *Desulfitobacterium* (*Firmicutes*, e.g. *Desulfitobacterium hafniense*) are generalists with a versatile metabolism [17, 18]. Numerous studies have reported OHR activity and occurrence of OHRB and *rdhA* genes in marine environments [6, 19-21]. Recent genomic

[22-24] and single-cell genomic [25] analyses revealed widespread occurrence of *rdh* gene clusters in marine *Deltaproteobacteria*, indicting untapped potential for OHR. Accordingly, OHR metabolism was experimentally verified in three *Deltaproteobacteria* strains, not previously known as OHRB [23].

OHRB, and in particular members of the *Chloroflexi*, are fastidious microbes, and are susceptible to inhibition by oxygen [26], sulfate [27] or sulfide [28, 29]. In the presence of both 3-chlorobenzoate and either sulfate, sulfite or thiosulfate, *Desulfomonile tiedjei* isolated from sewage sludge preferentially performed sulfur oxyanion reduction [30], and OHR inhibition was suggested to be caused by downregulation of *rdh* gene expression [30]. In contrast, concurrent sulfate reduction and OHR was observed in *Desulfoluna spongiiphila* AA1^T isolated from the marine sponge *Aplysina aerophoba* [20], and three newly characterized organohalide-respiring marine deltaproteobacterial strains [23]. Sulfate- and sulfide-rich marine environments may have exerted a selective pressure resulting in development of sulfate- and sulfide-tolerant OHRB.

The genus *Desulfoluna* comprises two anaerobic sulfate-reducing strains, *D. spongiiphila* AA1^T isolated from the bromophenol-producing marine sponge *Aplysina aerophoba* [20, 31], and *D. butyratoxydans* MSL71^T isolated from estuarine sediments [32]. Strain AA1^T can reductively dehalogenate various bromophenols but not chlorophenols. The genome of strain AA1^T harbours three *rdhA* genes, one of which was shown to be induced by 2,6-dibromophenol [21]. The OHR potential and the genome of strain MSL71^T have not been studied before. In this study, a third member of the genus *Desulfoluna*, designated *D. spongiiphila* strain DBB, was isolated from a marine intertidal sediment. The OHR metabolism of strain DBB and of strain MSL71^T was verified in this study. In line with former reports [22-25], this study further reinforces an important role of marine organohalide-respiring *Deltaproteobacteria* in halogen, sulfur and carbon cycling.

Materials and Methods

104 **Chemicals**

105 Brominated, iodinated and chlorinated benzenes and phenols were purchased
106 from Sigma-Aldrich. Other organic and inorganic chemicals used in this study were of
107 analytical grade.

108

109 **Bacterial strains**

110 *D. spongiiphila* AA1^T (DSM 17682^T) and *D. butyratoxydans* MSL71^T (DSM 19427^T)
111 were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ,
112 Braunschweig, Germany), and were cultivated as described previously [20, 32].

113

114 **Enrichment, isolation and cultivation of strain DBB**

115 Surface sediment of an intertidal zone, predominantly composed of shore
116 sediment, was collected at the shore in L'Escala, Spain (42°7'35.27"N 3°8'6.99"E). Five
117 grams of sediment were transferred into 120-ml bottles containing 50 ml of anoxic
118 medium [33] with lactate and 1,4-dibromobenzene (1,4-DBB) as the electron donor and
119 acceptor, respectively. Sediment-free cultures were obtained by transferring the
120 suspensions of the enrichment culture to fresh medium. A pure culture of a 1,4-DBB
121 debrominating strain, designated as *D. spongiiphila* strain DBB, was obtained from a
122 dilution series on solid medium with 0.8% low-melting point agarose (Sigma-Aldrich). A
123 detailed description of enrichment, isolation and physiological characterization of strain
124 DBB is provided in the Supplementary Information.

125

126 **Cell morphology and cellular fatty acids analyses**

127 Cell morphology and motility were observed using a LEICA DM 2000 Microscope
128 and a JEOL-6480LV Scanning Electron Microscope (SEM). Actively growing cells were
129 directly observed under the 100x magnification objective of the LEICA DM 2000
130 Microscope. Sample fixation and dehydration for SEM were performed as described
131 previously [34]. The cellular fatty acid composition was analysed from 500 ml cultures of
132 AA1^T, DBB and MSL71^T, which were grown with 20 mM lactate and 10 mM sulfate. Fatty

133 acids in the cell were analysed by acid hydrolysis of total cell material following a method
134 previously described [35].
135
136 **DNA extraction and bacterial community analysis**
137 DNA of the intertidal sediment (5 g) and the 1,4-DBB-respiring enrichment culture
138 (10 ml) was extracted using the DNeasy PowerSoil Kit (MO-BIO, CA, USA). A 2-step PCR
139 strategy was applied to generate barcoded amplicons from the V1—V2 region of bacterial
140 16S rRNA genes as described previously [36]. Sequence analysis was performed using
141 NG-Tax [37]. Operational taxonomic units (OTUs) were assigned taxonomy using uclust
142 [38] in an open reference approach against the SILVA 16S rRNA gene reference database
143 (LTPs128_SSU) [39]. Finally, a biological observation matrix (biom) file was generated
144 and sequence data were further analyzed using Quantitative Insights Into Microbial
145 Ecology (QIIME) v1.2 [40].
146
147 **Genome sequencing and annotation**
148 DNA of DBB and MSL71^T cells was extracted using the MasterPure™ Gram Positive
149 DNA Purification Kit (Epicentre, WI, USA). The genomes were sequenced using the
150 Illumina HiSeq2000 paired-end sequencing platform (GATC Biotech, Konstanz,
151 Germany). The genome of strain DBB was further sequenced by PacBio sequencing
152 (PacBio RS) to obtain longer read lengths. Optimal assembly kmer size for strain DBB
153 was detected using kmergenie (v.1.7039) [41]. A *de novo* assembly with Illumina
154 HiSeq2000 paired-reads was made with assembler Ray (v2.3.1) [41] using a kmer size of
155 81. A hybrid assembly for strain DBB with both the PacBio and the Illumina HiSeq reads
156 was performed with SPAdes (v3.7.1, kmer size: 81) [42]. The two assemblies were
157 merged using the tool QuickMerge (v1) [43]. Duplicated scaffolds were identified with
158 BLASTN [44] and removed from the assembly. Assembly polishing was performed with
159 Pilon (v1.21) [45] using the Illumina HiSeq reads. Optimal assembly kmer size for strain
160 MSL71^T was also identified using kmergenie (v.1.7039), and a *de novo* assembly with
161 Illumina HiSeq2000 paired-end reads was performed with SPAdes (v3.11.1) with a kmer-

size setting of 79,101,117. FastQC and Trimmomatic (v0.36) [46] was used for read inspection and trimming using the trimmomatic parameters: TRAILING:20 LEADING:20 SLIDINGWINDOW:4:20 MINLEN:50. Trimmed reads were mapped with Bowtie2 v2.3.3.1 [47]. Samtools (v1.3.1) [48] was used for converting the bowtie output to a sorted and indexed bam file. The assembly was polished with Pilon (v1.21).

Transcriptional analysis of the *rdhA* genes of *D. spongiiphila* DBB

Transcriptional analysis was performed using DBB cells grown with lactate (20 mM), sulfate (10 mM) and either 1,4-DBB (1 mM) or 2,6-DBP (0.2 mM). DBB cells grown with lactate and sulfate but without any organohalogens were used as control. Ten replicate microcosms were prepared for each experimental condition, and at each sampling time point, two microcosms were randomly selected and sacrificed for RNA isolation as described previously [49]. RNA was purified using RNeasy columns (Qiagen, Venlo, The Netherlands) followed by DNase I (Roche, Almere, The Netherlands) treatment. cDNA was synthesized from 200 ng total RNA using SuperScript™ III Reverse Transcriptase (Invitrogen, CA, USA) following manufacturer's instructions. RT-qPCR assays were performed as outlined in Supplementary Information.

Protein extraction and proteomic analysis

Triplicate cultures of strain DBB grown with lactate/sulfate (LS condition) or lactate/sulfate/1,4-DBB (LSD condition) were used for proteomic analysis. Preparation of cell-free extracts (CFE), determination of protein concentration, SDS-PAGE purification of total proteins in CFE and of proteins in membrane fragments, and the peptide fingerprinting-mass spectrometry (PF-MS) analysis, were performed as outlined in Supplementary Information. Statistical analysis was performed using prostar proteomics [50]. Top three peptide area values were normalized against all columns. The values of proteins detected in at least two of the three replicates were differentially compared and tested for statistical significance. Missing values were imputed using the SLSA function of prostar, and hypothesis testing with a student's t-test was performed for LSD vs LS

191 growth conditions. The *p*-values were Benjamini-Hochberg corrected and proteins with p-
192 values below 0.05 and a log2 value of 1 or larger were considered statistically
193 significantly up- or downregulated.
194
195 **Analytical methods**
196 Halogenated benzenes and benzene were analyzed on a GC equipped with an Rxi-
197 5Sil capillary column (Retek, PA, USA) and a flame ionization detector (GC-FID,
198 Shimadzu 2010). Halogenated phenols and phenol were analyzed on a Thermo Scientific
199 Accela HPLC System equipped with an Agilent Poroshell 120 EC-C18 column and a UV/Vis
200 detector. Organic acids and sugars were analyzed using a ThermoFisher Scientific
201 SpectraSYSTEM™ HPLC equipped with an Agilent Metacarb 67H column and RI/UV
202 detectors. Sulfate, sulfite and thiosulfate were analyzed using a ThermoFisher Scientific
203 Dionex™ ICS-2100 Ion Chromatography System equipped with a Dionex Ionpac
204 analytical column and a suppressed conductivity detector. Cell growth was determined by
205 measuring OD_{600} using a WPA CO8000 cell density meter (Biochrom, Cambridge, UK).
206 Sulfide was measured by a photometric method using methylene blue as described
207 previously [51].
208
209 **Strain and data availability**
210 *D. spongiiphila* strain DBB was deposited in DSMZ under accession number DSM
211 104433. The 16S rRNA gene sequences of strain DBB were deposited in GenBank
212 (accession numbers: MK881098—MK881099). The genome sequences of strains DBB and
213 MSL71 were deposited in the European Bioinformatics Institute (EBI, Project ID:
214 PRJEB31368). A list of proteins detected from strain DBB under LS and LDS growth
215 conditions is available in Supplementary Datasets 1 (Soluble fraction) and 2 (Membrane
216 fraction).
217
218 **Results and discussion**
219

220 **Enrichment of 1,4-DBB debrominating cultures and isolation of strain DBB**

221 Reductive debromination of 1,4-DBB to bromobenzene (BB) and benzene was
222 observed in the original cultures containing intertidal sediment (Fig. 1A, 1B).
223 Debromination of 1,4-DBB was maintained in the subsequent sediment-free transfer
224 cultures (Fig. 1C). However, benzene was no longer detected and BB was the only
225 debromination product, indicating loss of the BB-debrominating population. Up to date,
226 the only known OHRB that can debrominate BB to benzene is *Dehalococcoides mccartyi*
227 strain CBDB1 [52]. 1,4-DBB debromination to BB was stably maintained during
228 subsequent transfers (data not shown) and after serial dilution (Fig. 1D). Bacterial
229 community analysis showed an increase in the relative abundance of *Deltaproteobacteria*
230 from ~2% in the intertidal sediment at time zero to ~13% after 104 days of enrichment
231 (Fig. 1E). The genus *Desulfoluna* was highly enriched and comprised more than 80%
232 relative abundance in the most diluted culture (10^7 dilution) (Fig. 1E).

233 Single colonies were observed in roll tubes with 0.8% low-melting agarose after
234 15 days of incubation. Among the six single colonies randomly selected and transferred
235 to liquid media, one showed 1,4-DBB debromination (Fig. 1F) which was again subjected
236 to the roll tube isolation procedure to ensure purity. The final isolated strain was
237 designated DBB.

238

239 **Characterization of the *Desulfoluna* strains**

240 Cells of strain DBB were slightly curved rods with a length of 1.5 to 3 µm and a
241 diameter of 0.5 µm as revealed by SEM (Fig. S1A, S1B), which was similar to strain AA1^T
242 (Fig. S1C) and MSL71^T (Fig. S1D). In contrast to strain AA1^T [20], but similar to strain
243 MSL71^T [32], strain DBB was motile when observed by light microscopy, with evident
244 flagella being observed by SEM (Fig. S1A, B).

245 The cellular fatty acid profiles of the three strains consisted mainly of even-
246 numbered saturated and mono-unsaturated fatty acids (Table S2).

247 Strain DBB used lactate, pyruvate, formate, malate and butyrate as electron
248 donors for sulfate reduction (Table 1). Lactate was degraded to acetate, which

accumulated without further degradation, and sulfate was reduced to sulfide (Fig. S1A). In addition, sulfite and thiosulfate were utilized as electron acceptors with lactate as the electron donor (Table 1). Sulfate and 1,4-DBB could be concurrently utilized as electron acceptors by strain DBB (Fig. S2). Independent of the presence of sulfate in the medium, strain DBB stoichiometrically debrominated 1,4-DBB to bromobenzene (BB), and 2-bromophenol (2-BP), 4-bromophenol (4-BP), 2,4-bromophenol (2,4-DBP), 2,6-DBP, 2,4,6-tribromophenol (2,4,6-TBP), 2-iodophenol (2-IP) and 4-iodophenol (4-IP) to phenol (Table 1) using lactate as the electron donor. Hydrogen was not used as an electron donor for 1,4-DBB debromination (data not shown). Strain DBB was unable to dehalogenate the tested chlorinated aromatic compounds and several other bromobenzenes listed in Table 1. This is in accordance with the dehalogenating activity reported for strain AA1^T that was unable to use chlorinated aromatic compounds as electron acceptors [20]. The majority of the known organohalogens from marine environments are brominated [1] and hence marine OHRB may be less exposed to organochlorine compounds in their natural habitats. For instance, strain AA1^T was isolated from the sponge *Aplysina aerophoba* [20] in which organobromine metabolites can account for over 10% of the sponge dry weight [53].

Genomic and phylogenetic characterization of the *Desulfoluna* strains

The genome of strain DBB is closed and consists of a single chromosome with a size of 6.68 Mbp (Fig. S3). The genome of strain AA1^T (GenBank accession number: NZ_FMUX01000001.1) and strain MSL71T (sequenced in this study) are draft genomes with similar G+C content (Table 1). The average nucleotide identity (ANI) of the DBB genome to AA1^T and MSL71^T genomes was 98.5% and 85.9%, respectively. This indicates that DBB and AA1^T strains belong to the same species of *D. spongiiphila* [54]. 16S rRNA gene and protein domain-based phylogenetic analyses with other genera of the *Desulfobacteraceae* placed *Desulfoluna* strains in a separate branch of the corresponding phylogenetic trees (Fig. 2). Whole genome alignment of strains DBB, AA1^T and MSL71^T revealed the presence of 11 locally colinear blocks (LCBs) with several small regions of

278 inversion and rearrangement (Fig. S4). A site-specific recombinase gene (DBB_14420)
279 was found in one of the LCBs. The same gene was also found in the corresponding
280 inversed and rearranged LCBs in AA1^T (AA1_11599) and MSL71^T (MSL71_ 48620),
281 suggesting a role of the encoded recombinase in genomic rearrangement in the
282 *Desulfoluna* strains.
283
284 **Comparison of the *rdh* gene region of the *Desulfoluna* strains**
285 Similar to strain AA1^T [21], the genomes of strains DBB and MSL71^T also harbor
286 three *rdhA* genes. The amino acid sequences of the RdhA homologs in DBB share >99%
287 identity to the corresponding RdhAs in AA1^T, and 80—97% identity with the
288 corresponding RdhAs in MSL71^T (Fig 3). However, the three distinct RdhA homologs in
289 the *Desulfoluna* strains share low identity (20—30%) with each other and form three
290 distant branches in the phylogenetic tree of RdhAs [18], and cannot be grouped with any
291 of the currently known RdhA groups (Fig. S5). Therefore, we propose three new RdhA
292 homolog groups, RdhA1 including DBB_38400, AA1_07176 and MSL71_22580; RdhA2
293 including DBB_36010, AA1_02299 and MSL71_20560; RdhA3 including DBB_45880,
294 AA1_11632 and MSL71_30900 (Fig 3, Fig. S5).
295 The *rdh* gene clusters in DBB and MSL71^T show a similar gene order to the
296 corresponding *rdh* gene clusters in AA1^T (Fig. 3), except that the *rdhA1* gene cluster of
297 MSL71^T lacks *rdhB* and *rdhC*. Genes encoding sigma-54-dependent transcriptional
298 regulators in the *rdhA1* and *rdhA3* gene clusters of AA1^T [21], were also present in the
299 corresponding gene clusters of DBB and MSL71^T (Fig. 3). Likewise, genes encoding the
300 LuxR and MarR-type regulators are present up- and downstream of the *rdhA2* gene
301 clusters of DBB and MSL71^T, in line with the organization of the *rdhA2* gene cluster of
302 AA1^T (Fig. 3). This may indicate similar regulation systems of the *rdh* genes in the
303 *Desulfoluna* strains studied here. The conserved motifs from known RDases (RR, C1−C5,
304 FeS1, and FeS2) [55, 56] are also conserved among all the RdhAs of the *Desulfoluna*
305 strains, except for RdhA1 of MSL71^T which lacks the RR motif (Fig. S6). This may indicate
306 a cytoplasmic localization and a non-respiratory role of the RdhA1 in strain MSL71^T [6].

307

308 **OHR metabolism of *D. butyratoxydans* MSL71^T**

309 Guided by the genomic potential of strain MSL71^T for OHR, physiological
310 experiments in this study indeed confirmed that strain MSL71^T is capable of using 2-BP,
311 2,4-DBP, 2,6-DBP and 2,4,6-TBP as electron acceptors with lactate as the electron donor.
312 Similar to DBB and AA1^T, chlorophenols such as 2,4-DCP, 2,6-DCP and 2,4,6-TCP were
313 not dehalogenated by strain MSL71^T (Table 1). In contrast to strains DBB and AA1^T,
314 strain MSL71^T was unable to debrominate 1,4-DBB and 4-BP. Hence, debromination of
315 2,4-DBP and 2,4,6-TBP was incomplete with 4-BP as the final product rather than phenol
316 (Table 1). Moreover, strain MSL71^T was unable to deiodinate 2-IP and 4-IP, again in
317 contrast to strains DBB and AA1^T (Fig. S7, Table 1).

318

319 **Induction of *rdhA* genes during OHR by strain DBB**

320 When strain DBB was grown with sulfate and 1,4-DBB with concomitant
321 production of BB (Fig. 4A), its *rdhA1* gene showed significant up-regulation (60-fold) at
322 24 h, reached its highest level (120-fold) at 48 to 72 h, and then decreased (Fig. 4B). In
323 contrast, no significant up-regulation of *rdhA2* or *rdhA3* was noted, suggesting that
324 RdhA1 mediates 1,4-DBB debromination. Accordingly, RdhA1 was found in the proteome
325 of the LSD growth condition but not in that of the LS condition (Table S4, Dataset S1 and
326 S2). When strain DBB was grown with sulfate and 2,6-DBB, both *rdhA1* and *rdhA3* were
327 significantly up-regulated and reached their highest level at 4 h (65- and 2000-fold,
328 respectively, Fig. 4D). However, *rdhA3* was the dominant gene at 8 h (Fig. 4D), after
329 which 2-BP was debrominated to phenol (Fig. 4C) indicating a role of RdhA3 in 2,6-DBP
330 and 2-BP debromination by strain DBB. A previous transcriptional study of the *rdhA*
331 genes in strain AA1^T during 2,6-DBP debromination also showed a similar induction of its
332 *rdhA3* [21].

333

334 **Corrinoid biosynthesis in *Desulfoluna* strains**

Most known RDases depend on corrinoid cofactors such as cyanocobalamin for dehalogenation activity [10]. Both strains DBB (this study) and AA1^T [21] were capable of OHR in the absence of externally added cobalamin. With one exception (*cbiJ*), the genomes of the *Desulfoluna* strains studied here harbor all genes necessary for *de novo* anaerobic corrinoid biosynthesis starting from glutamate (Table S5). The genes for cobalamin biosynthesis from precorrin-2 are arranged in one cluster (DBB_3730—3920, AA1_12810—12829, MSL71_49290—49480) including an ABC transporter (*btuCDF*) for cobalamin import (Fig. 5). Another small cobalamin-related gene cluster was detected in the *Desulfoluna* genomes (DBB_52170—52260, AA1_10815—10826, MSL71_44540—44630), which includes genes coding for the outer membrane corrinoid receptor BtuB and a second copy of the corrinoid-transporter BtuCDF plus another BtuF. Additionally, cobaltochelatase CbiK as well as a putative cobaltochelatase CobN are encoded in this gene cluster. The latter is usually involved only in the aerobic cobalamin biosynthesis pathway, and its function in *Desulfoluna* strains is unknown. Three of the proteins encoded by DBB_3730—3920 (Cbik: 3730, CbiL: 3790, CbiH: 3850) were detected in the proteome of cells grown under both the LS and LSD conditions (Table S4, Dataset S1). The abundance of the cobalamin biosynthesis proteins was not significantly different between LS and LSD conditions (Table S4, Dataset S1 and S2), except for the tetrapyrrole methylase CbiH encoded by DBB_3850 that was significantly more abundant in LSD cells (Table S4, Dataset S1). The detection of cobalamin biosynthesis proteins in the absence of 1,4-DBB in LS condition could be due to the synthesis of corrinoid-dependent enzymes in the absence of an organohalogen. Accordingly, three corrinoid-dependent methyltransferase genes (encoded by DBB_7090, 43520, 16050) were detected in the proteomes, which might be involved in methionine, methylamine or o-demethylation metabolism. This might also indicate a constitutive expression of the corresponding genes, in contrast to the organohalide-induced cobalamin biosynthesis in *Sulfurospirillum multivorans* [57].

363 **Sulfur metabolism and impact of sulfate and sulfide on debromination by**
364 ***Desulfoluna* strains**

365 All three strains were capable of using sulfate, sulfite, and thiosulfate as the
366 terminal electron acceptors (Table 1). Four sulfate permease genes are present in the
367 genomes of the *Desulfoluna* strains (Table S6), and one of the sulfate permeases
368 (DBB_22290) was detected in DBB cells grown under LS and LSD conditions (Table S4,
369 Dataset S2). The genes involved in sulfate reduction, including those encoding sulfate
370 adenylyltransferase (Sat), APS reductase (AprBA) and dissimilatory sulfite reductase
371 (DsrAB), were identified in the genomes of all three strains (Table S6). The
372 corresponding proteins were detected in DBB cells grown under both LS and LDS
373 conditions (Fig. 6, Table S4) with AprBA, disulfite reductase (DsrMKJOP) and Sat among
374 the most abundant proteins in both, soluble and membrane fractions (Dataset S1 and
375 S2). Tetrathionate reductase encoding genes (*ttrA*) were found only in the genomes of
376 strains DBB and AA1^{T}. Interestingly, thiosulfate reductase genes were not found in any of
377 the three genomes, whereas all strains can use thiosulfate as the electron acceptor
378 (Table 1). *Desulfitobacterium metallireducens* was also reported to reduce thiosulfate
379 despite lacking a known thiosulfate reductase gene [58, 59], suggesting the existence of
380 a not-yet-identified gene encoding a thiosulfate reductase [59]. Possible alternatives are
381 genes encoding rhodanese-like protein (RdlA) (Table S6) [60] or the three-subunit,
382 periplasmic molybdopterin oxidoreductase (Table S6), as a putative polysulfide reductase
383 (Psr) [61].

384 Sulfate and sulfide are known inhibitors of many OHRB [30, 62, 63]. However,
385 debromination of 2,6-DBP was not affected in *Desulfoluna* strains in the presence of up to
386 20 mM sulfate (Fig. S8B, D, F), and sulfate and 2,6-DBP were reduced concurrently (Fig.
387 S8). This is similar to some other *Deltaproteobacteria* [23], but in contrast to *D. tiedjei*
388 which preferentially performs sulfate reduction over OHR with concomitant down-
389 regulation of *rdh* gene expression [30]. Moreover, sulfide, an RDase inhibitor in *D. tiedjei*
390 [64] and *Dehalococcoides mccartyi* strains [28, 29], did not impact 2,6-DBP
391 debromination by *Desulfoluna* strains at a concentration of 10 mM (Fig. S9A—F).

392 However, debromination was delayed in the presence of 20 mM sulfide, and no
393 debromination was noted in the presence of 30 mM sulfide (Fig. S9G—L). This high
394 resistance to sulfide was not reported before for the known OHRB, and is also rare
395 among sulfate-reducing bacteria [65], and may confer an ecological advantage to these
396 sulfate-reducing OHRB. Although hydrogen sulfide can be oxidized abiotically or serve as
397 electron donor for sulfide-oxidizing microorganisms [66], naturally sustained and high
398 concentrations of hydrogen sulfide are found in some marine environments [67].
399
400 **Electron transport chains of strain DBB**
401 Two lactate dehydrogenases (LdhA-1/2, DBB_24880/24970) with HdrD-like
402 putative iron-sulfur subunits (LdhB-1/2, DBB_24870/24960) were found in the proteome
403 of DBB cells grown under LS and LSD conditions. Similar Ldhs were reported to be
404 essential for the growth of *Desulfovibrio alaskensis* G20 with lactate and sulfate [68].
405 Similar to *D. alaskensis* G20 and *D. vulgaris* strain Hildenborough [68, 69], the two Ldhs
406 were encoded by an organic acid oxidation gene cluster (DBB_24870—24970) including
407 genes encoding lactate permease (DBB_24890), the Ldhs and pyruvate oxidoreductase
408 (Por, DBB_24940). Based on previous studies with *D. vulgaris* Hildenborough [70], the
409 electron transport pathway in strain DBB with lactate and sulfate could take one of the
410 following routes: the Ldh's either reduce menaquinone directly [70], or transfer electrons
411 via the HdrD-like subunit [71] and DsrC (DBB_370, a high redox potential electron
412 carrier with disulfide/dithiol (RSS/R(SH)2)) to QmoA [72]. The pyruvate produced by
413 lactate oxidation is further oxidized by Por (DBB_310/24940), and the released electrons
414 are carried/transferred by a flavodoxin (DBB_37290). From there, the electrons from the
415 low-potential ferredoxin and the electrons from the high-potential (disulfide bond) DsrC
416 could be confurcated to QmoABC, which reduces menaquinone (Fig. 6A, B). The electrons
417 are then transferred from menaquinol to the APS reductase (AprBA, DBB_23880—890)
418 which is, together with three other enzyme complexes (Sat, encoded by DBB_23930,
419 DsrABD, DBB_25620—640, and DsrMKJOP, DBB_27290—330), responsible for the sulfate
420 reduction cascade (Santos et al., 2015).

421 The electron transport chain from Ldh to menaquinones or QmoABC during OHR is
422 likely shared with sulfate reduction. Electron transport from menaquinol ($E^{0\prime}$ = -75 mV)
423 to the RDase ($E^{0\prime}$ (CoII/CoI) ≈ -360 mV) is thermodynamically unfavorable (Schubert et
424 al., 2018), and the proteins involved to overcome this barrier have not been identified
425 and most likely are not the same in different organohalide-respiring bacterial genera.
426 Based on the genomic and proteomic analyses of strain DBB, we identified several
427 possible electron transfer proteins connecting the menaquinone pool and RdhA1. The first
428 is the membrane-integral protein RdhC1 (encoded by DBB_38380, Fig. 3), a homolog of
429 proteins previously proposed to function as transcriptional regulator for *rdhAB* gene
430 expression in *Desulfitobacterium dehalogenans* [73]. However, a recent study on PceC
431 from *Dehalobacter restrictus* proposed a possible role for RdhC in electron transfer from
432 menaquinones to PceA via its exocytoplasmically-facing flavin mononucleotide (FMN) co-
433 factor [16]. RdhC in *Desulfoluna* strains also showed the conserved FMN binding motif (in
434 particular the fully conserved threonine residue) and two CX_3CP motifs predicted to have
435 a role in electron transfer [16] (Fig. S10). Moreover, the five transmembrane helices of
436 RdhC in DBB were also conserved (Fig. S11), indicating a possible function of RdhC1 in
437 electron transfer from menaquinones to RdhA1 (Fig. 6A). However, RdhC1 was not found
438 in our proteomic analysis, probably due to tight interaction with the membrane.
439 A second link between menaquinol/QmoABC and RdhA1 could be the Fix complex
440 homolog, an electron transfer flavoprotein complex found in nitrogen-fixing
441 microorganisms such as *Azotobacter vinelandii* and *Rhodospirillum rubrum* [74, 75]. The
442 Fix complex is capable of using electron bifurcation to generate low-potential reducing
443 equivalents for nitrogenase [74]. Strain DBB does not encode the minimum genes
444 necessary for nitrogen fixation [76]. Hence, the Fix complex in DBB cells is likely linked
445 to other cellular processes. Induction of the *fix* genes under OHR conditions was reported
446 in other OHRB such as *Desulfitobacterium hafniense* TCE1 [77], and the corresponding
447 Fix complex was suggested to provide low-redox-potential electrons for OHR. However,
448 the obligate organohalide-respiring *Dehalobacter* spp., which are phylogenetically related
449 to *Desulfitobacterium* spp., do not encode FixABC, questioning a general role of Fix

complex in OHR (Türkowsky et al., 2018). In strain DBB, the abundance of FixABC (encoded by DBB_25970—990) was not higher in the cells grown under LDS as opposed to LS condition, but FixAB were among the most abundant 10% proteins in the soluble fraction (Dataset S1), indicating a potential role in electron transfer in both sulfate reduction and OHR. In this scenario, FixABC accepts two electrons from menaquinol, subsequently bifurcating them to unidentified high- and low-potential electron acceptors (Fig. 6B). The low-potential electron acceptor may also serve as an electron carrier that transfers electrons from cytoplasm-facing FixABC to the exoplasm-facing RdhA1 via an as-yet-unidentified electron carrier across the membrane [78] (Fig. 6B).

A third scenario is the involvement of QmoABC- and QrcABCD-mediated reverse electron transport (Fig. 6C), similar to the electron transport system of *D. alaskensis* G20 cultivated in syntrophic interaction with *Methanococcus maripaludis* [68]. The electron transport from menaquinol to the periplasmic hydrogenase or formate dehydrogenase in strain G20 also needs to overcome an energy barrier similar to that of OHR (redox potential of H_2/H^+ and formate/CO_2 are −414 mV and −432 mV, respectively) [68]. In this scenario, lactate is oxidized to pyruvate as described above, transferring electrons to a thiol-disulfide redox pair. Pyruvate is oxidized by Por and the electrons are accepted by the flavodoxin. QmoABC then confurcates electrons from the low-potential ferredoxin and the high-potential thiol-disulfide redox pair to drive reduction of menaquinones. Electrons are transferred from menaquinol to RdhA1 via QrcABCD by reverse electron transport (Fig. 6C). The energy required for reverse electron transport is likely derived from the proton motive force mediated by QrcABCD [79]. In this scenario, QmoABC plays a key role in the metabolism of strain DBB as a link between sulfate reduction and OHR. This electron transport pathway provides a possible explanation for the increased 1,4-DBB debromination rate by DBB when sulfate is concurrently present (Fig. 1E, Fig. S1B). Hence, sulfate reduction may stimulate the electron confurcation process that is also used for OHR. Moreover, sulfate reduction can generate the proton motive force required for the reverse electron transport from QmoABC to RdhA1. Qmo and Qrc complexes are frequently found in sulfate-reducing *Deltaproteobacteria* and were proposed to be

479 involved in energy conservation [71, 80, 81]. However, biochemical studies with sulfate-
480 reducing OHRB are necessary to further corroborate such a reverse electron flow and the
481 intricate relationship of electron transfer in sulfate reduction and OHR.
482
483 **Potential oxygen defense in *Desulfoluna* strains**
484 Sulfate reducers, which have been assumed to be strictly anaerobic bacteria, not
485 only survive oxygen exposure but also can utilize it as an electron acceptor [82, 83].
486 However, the response of organohalide-respiring sulfate reducers to oxygen exposure is
487 not known. Most of the described OHRB are strict anaerobes isolated from anoxic and
488 usually organic matter-rich subsurface environments [17]. In contrast, strain DBB was
489 isolated from marine intertidal sediment mainly composed of shore sand (Fig. 1A), where
490 regular exposure to oxic seawater or air can be envisaged. The genomes of the
491 *Desulfoluna* strains studied here harbor genes encoding enzymes for oxygen reduction
492 and reactive oxygen species (ROS) detoxification (Table S7). Particularly, the presence of
493 a cytochrome *c* oxidase is intriguing and may indicate the potential for oxygen
494 respiration. Accordingly, in the presence of 2% oxygen in the headspace of DBB cultures,
495 the redox indicator resazurin in the medium turned from pink to colorless within two
496 hours, indicating consumption/reduction of oxygen by strain DBB. Growth of strain DBB
497 on lactate and sulfate was retarded in the presence of 2% oxygen (Fig. S12C). However,
498 in both the presence (Fig. S12C) and absence of sulfate (Fig. S12D), slower but complete
499 debromination of 2,6-DBP to phenol was achieved with 2% oxygen in the headspace.
500 Neither growth nor 2,6-DBP debromination was observed with an initial oxygen
501 concentration of 5% in the headspace (Fig. S12E, F). Such resistance of marine OHRB to
502 oxygen may enable them to occupy niches close to halogenating organisms/enzymes
503 that nearly all use oxygen or peroxides as reactants [84]. For instance, the marine
504 sponge *A. aerophoba* from which *D. spongiiphila* AA1^T was isolated [20] harbors bacteria
505 with a variety of $FADH_2$-dependent halogenases [85], and produces a variety of
506 brominated secondary metabolites [53]. Testing survival and OHR of *Desulfoluna* strains
507 under continuous oxygen exposure and studying the mechanisms of oxygen defense as

508 studied in *Sulfurospirillum multivorans* [11] are necessary to further unravel oxygen
509 resistance/metabolism mechanisms in *Desulfoluna* strains.
510
511 **Conclusions**
512 Widespread environmental contamination with organohalogen compounds and
513 their harmful impacts to human and environmental health has been the driver of chasing
514 OHRB since the 1970s. In addition, the environment itself is an ample and ancient source
515 of natural organohalogens, and accumulating evidence shows widespread occurrence of
516 *rdhA* in marine environments [6]. The previous isolation and description of strain AA1^T
517 from a marine sponge, the isolation of strain DBB from intertidal sediment samples, and
518 verification of the OHR potential of strain MSL71^T in this study indicate niche
519 specialization of the members of the genus *Desulfoluna* as chemoorganotrophic
520 facultative OHRB in marine environments rich in sulfate and organohalogens. As such, *de*
521 *novo* corrinoid biosynthesis, resistance to sulfate, sulfide and oxygen, versatility in using
522 electron donors, and the capacity for concurrent sulfate and organohalogen respiration
523 confer an advantage to *Desulfoluna* strains in marine environments. Interestingly,
524 approximately 10% of the sequenced deltaproteobacterial genomes, that have mostly
525 been obtained from marine environments, contain one or multiple *rdh* genes [22, 23],
526 and OHR metabolism was experimentally verified in three strains not previously known
527 as OHRB [23]. These findings reinforce an important ecological role of sulfate-reducing
528 organohalide-respiring *Deltaproteobacteria* in sulfur, halogen and carbon cycling in a
529 range of marine environments.
530
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Figure legends

Figure 1. Enrichment and isolation of *D. spongiiphila* DBB. Intertidal sediment mainly composed of shore sediment used for isolation (A). Reductive debromination of 1,4-DBB by: the original microcosms containing intertidal sediment (B), the sediment-free enrichment cultures (C), the most diluted culture (10^7) in the dilution series (D). Phylogenetic analysis of bacterial communities in the microcosms from the shore sediment at time zero (left), the original 1,4-DBB debrominating enrichment culture after 104 days incubation (middle) and the 10^7 dilution series culture (right) (E). Reductive debromination of 1,4-DBB by the isolated pure culture (F). Sediment enrichment culture and sediment-free transfer cultures (B—D) were prepared in single bottles. Pure cultures (F) were prepared in duplicate bottles. Points and error bars represent the average and standard deviation of samples taken from the duplicate cultures. Phylogenetic data are shown at phylum level, except *Deltaproteobacteria* shown at class level and *Desulfoluna* at genus level. Taxa comprising less than 1% of the total bacterial community are categorized as 'Others'.

Figure 2. Phylogenetic tree based on 16S rRNA gene sequence and protein domain analyses. A comparison is included as horizontal lines between the two trees, showing the position of strain DBB relative to other strains belonging to the family *Desulfobacteraceae* as well as several *Desulfovibrio* strains. The "unique" nodes between the 16S rRNA gene- and domain-based tree are indicated with dashed lines. Genomes (Table S3) were selected based on the phylogenetic tree of the family *Desulfobacteraceae* [86].

564 **Figure 3.** Comparison of the *rdh* gene clusters in *D. spongiiphila* DBB, *D. spongiiphila*
565 AA1^T and *D. butyratoxydans* MSL71^T. Numbers indicate the locus tags of the respective
566 genes.
567 **Figure 4.** Debromination of 1,4-DBB (A) and 2,6-DBP (C) by *D. spongiiphila* DBB and
568 relative induction of its three *rdhA* genes during debromination of 1,4-DBB (B) and 2,6-
569 DBP (D). Error bars in panels A and C indicate the standard deviation of two random
570 cultures analyzed out of 10 replicates. The concentration of 1,4-DBB (> 0.1 mM) could
571 not be accurately measured due to large amount of undissolved compound and hence
572 was not plotted. Error bars in panels B and D indicate standard deviation of triplicate RT-
573 qPCRs performed on samples withdrawn from duplicate cultures at each time point (n =
574 2 × 3).
575 **Figure 5.** Corrinoid biosynthesis and transporter gene clusters of *Desulfoluna* strains.
576 Numbers indicate the locus tags of the respective genes. The corresponding enzymes
577 encoded by the genes and their functions in corrinoid biosynthesis are indicated in Table
578 S4.
579 **Figure 6.** Proposed electron transport pathways with OHR mediated by RdhC (A), Fix
580 complex (B), Qmo/Qrc complexes (C) in *D. spongiiphila* DBB grown on lactate and sulfate
581 (LS) and lactate, sulfate and 1,4-DBB (LSD). Corresponding gene locus tags are given for
582 each protein. Log protein abundance ratios between LSD and LS grown cells are indicated
583 next to the gene locus tag. Proteins shown in dashed line square were not detected
584 under the tested conditions. Probable electron flow path is shown in red arrows, and the
585 dashed red arrows indicate reverse electron transport.
586
587

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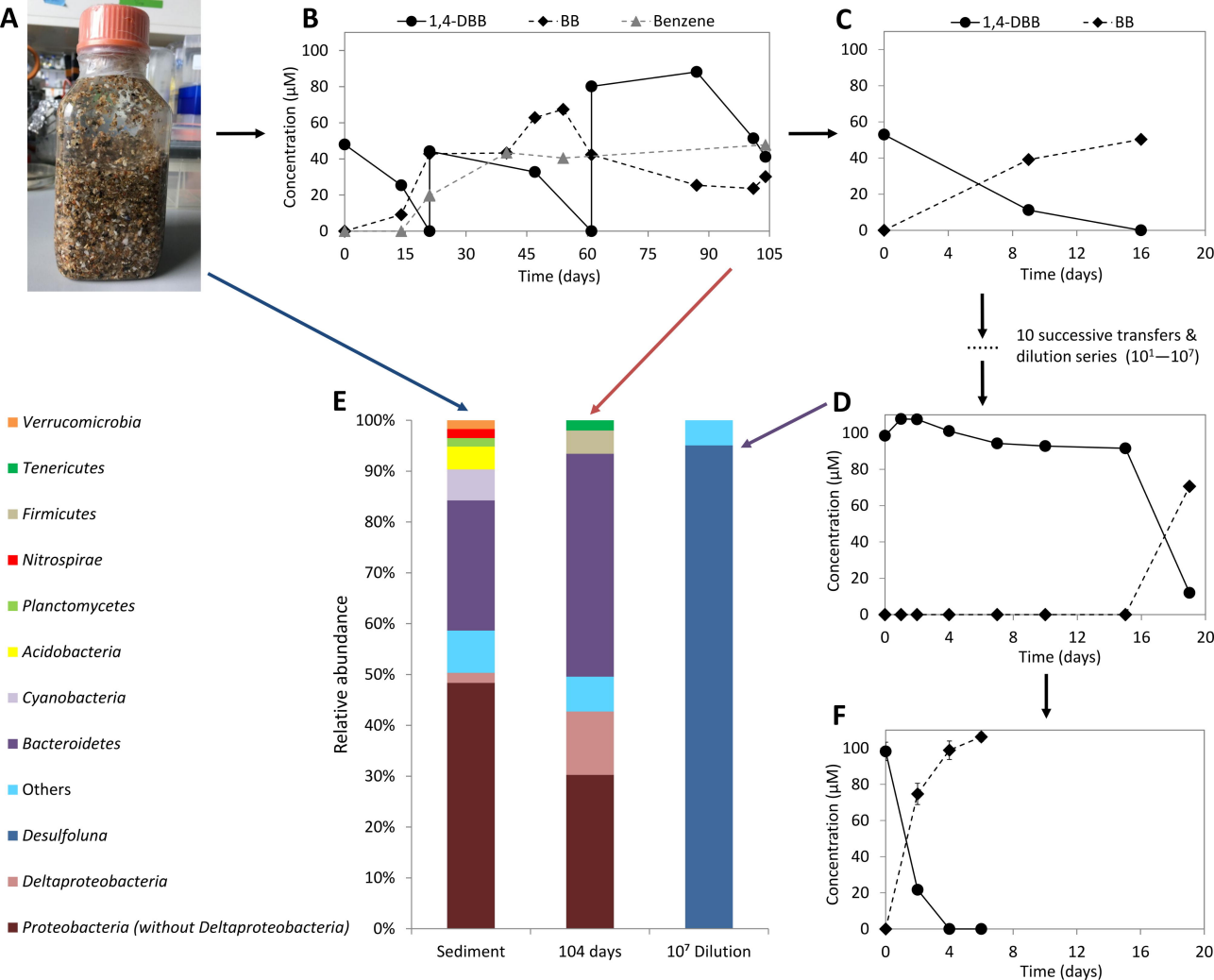
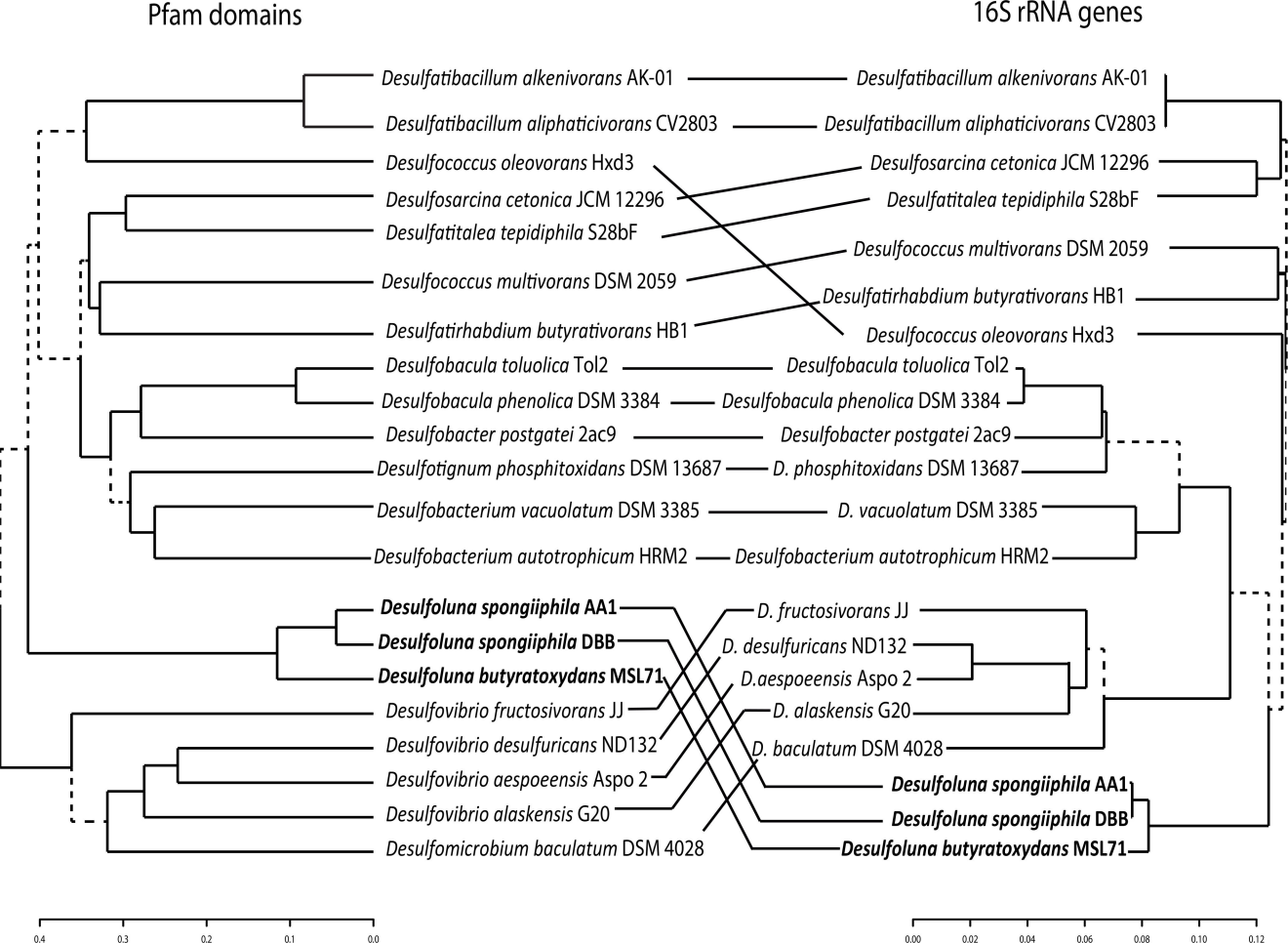

A
B
1,4-DBB
BB
Benzene
Concentration (μM)
Time (days)
C
1,4-DBB
BB
Time (days)
10 successive transfers & dilution series (10¹—10⁷)
D
Concentration (μM)
Time (days)
E
Relative abundance
Sediment
104 days
10⁷ Dilution
Verrucomicrobia
Tenericutes
Firmicutes
Nitrospirae
Planctomycetes
Acidobacteria
Cyanobacteria
Bacteroidetes
Others
Desulfoluna
Deltaproteobacteria
Proteobacteria (without Deltaproteobacteria)
F
Concentration (μM)
Time (days)


Pfam domains
16S rRNA genes
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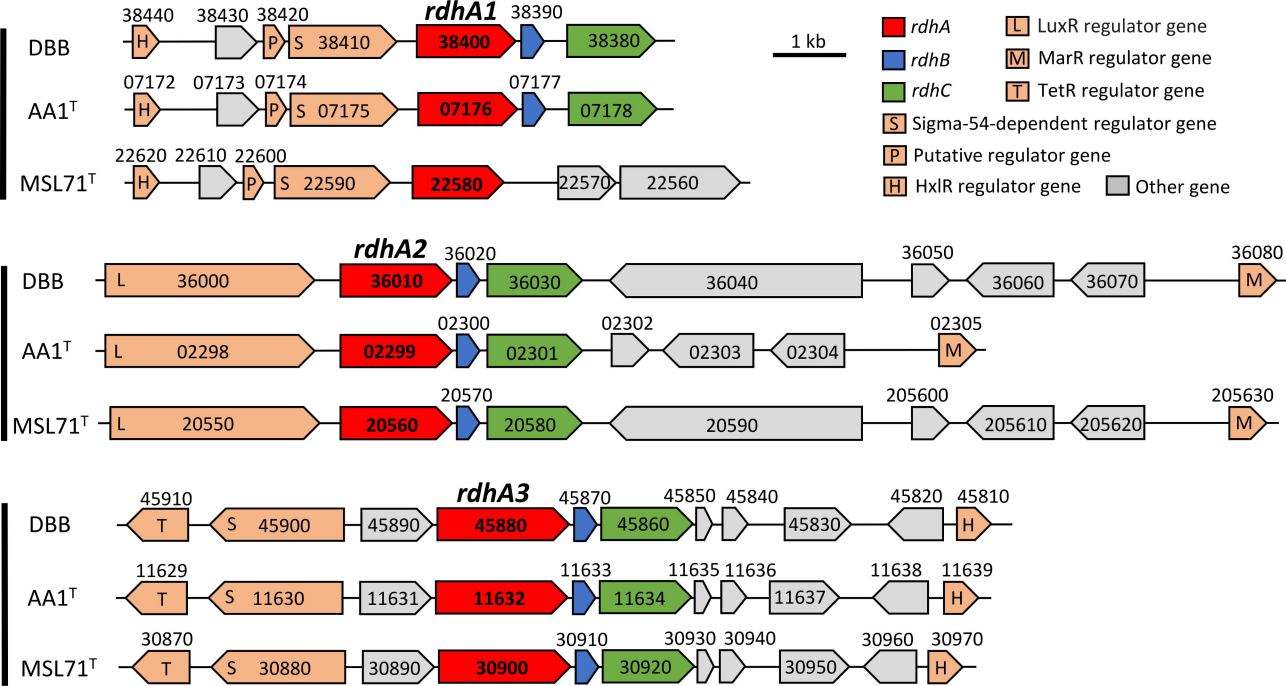
Desulfatibacillum aliphaticivorans CV2803
Desulfococcus oleovorans Hxd3
Desulfosarcina cetonica JCM 12296
Desulfatitalea tepidiphila S28bF
Desulfococcus multivorans DSM 2059
Desulfatirhabdium butyrativorans HB1
Desulfobacula toluolica Tol2
Desulfobacula phenolica DSM 3384
Desulfobacter postgatei 2ac9
Desulfotignum phosphitoxidans DSM 13687
Desulfobacterium vacuolatum DSM 3385
Desulfobacterium autotrophicum HRM2
Desulfoluna spongiiphila AA1
Desulfoluna spongiiphila DBB
Desulfoluna butyratoxydans MSL71
Desulfovibrio fructosivorans JJ
Desulfovibrio desulfuricans ND132
Desulfovibrio aespoeensis Aspo 2
Desulfovibrio alaskensis G20
Desulfomicrobium baculatum DSM 4028
D. fructosivorans JJ
D. desulfuricans ND132
D.aespoeensis Aspo 2
D. alaskensis G20
D. baculatum DSM 4028
0.4
0.3
0.2
0.1
0.0
0.00
0.02
0.04
0.06
0.08
0.10
0.12


rdhA1
38440
38430
38420
38390
DBB
H
P
S 38410
38400
38380
AA1T
07172
07173
07174
07177
H
P
S 07175
07176
07178
MSL71T
22620
22610
22600
H
P
S 22590
22580
22570
22560
1 kb
rdhA
rdhB
rdhC
L LuxR regulator gene
M MarR regulator gene
T TetR regulator gene
S Sigma-54-dependent regulator gene
P Putative regulator gene
H HxlR regulator gene
Other gene
rdhA2
36020
36050
36080
DBB
L 36000
36010
36030
36040
36060
36070
M
AA1T
02300
02302
02305
L 02298
02299
02301
02303
02304
M
MSL71T
20570
205600
205630
L 20550
20560
20580
20590
205610
205620
M
rdhA3
45910
45870
45850
45840
45820
45810
DBB
T
S 45900
45890
45880
45860
45830
H
AA1T
11629
11633
11635
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11638
11639
T
S 11630
11631
11632
11634
11637
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S 30880
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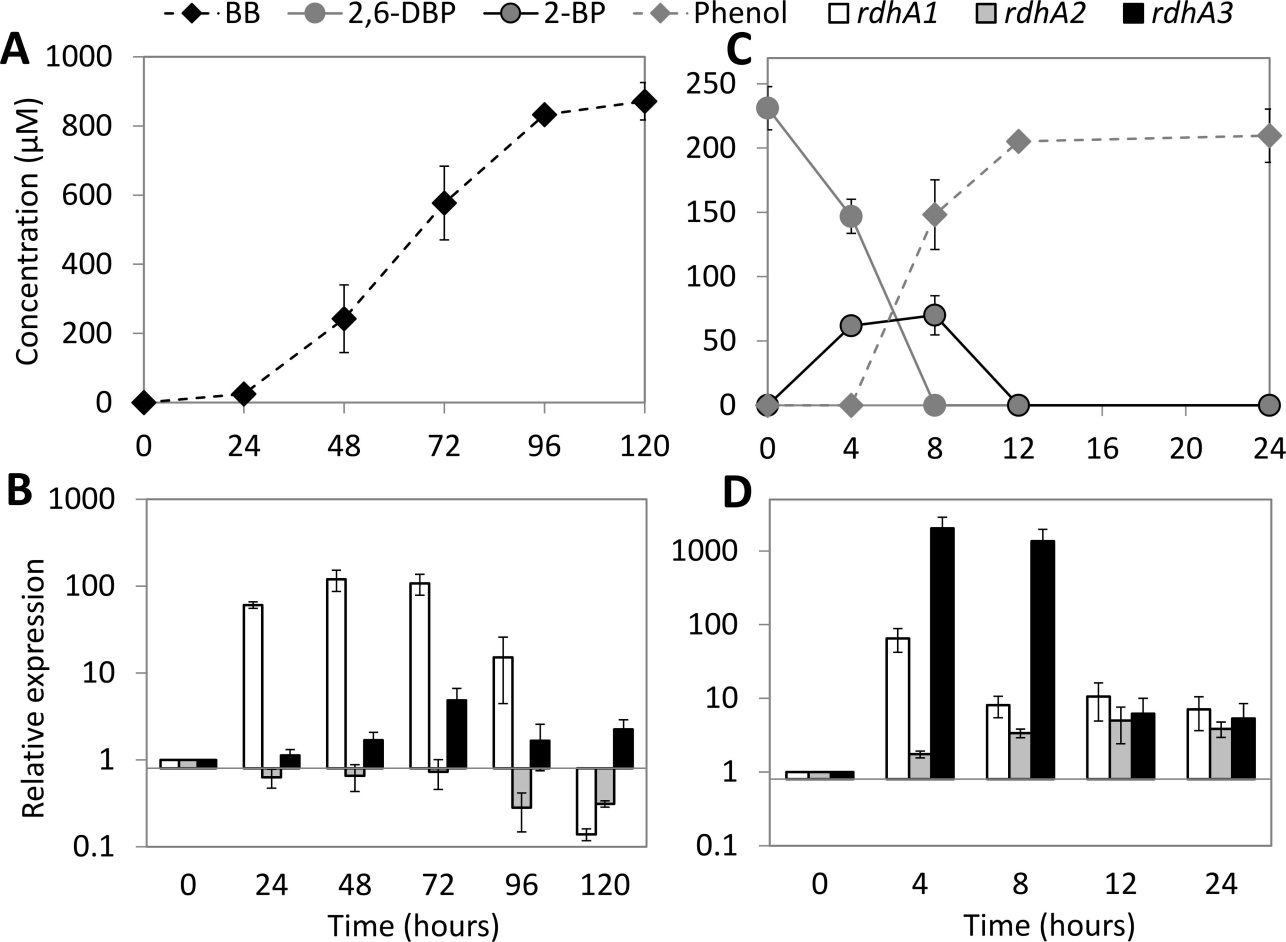
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2-BP
Phenol
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rdhA2
rdhA3
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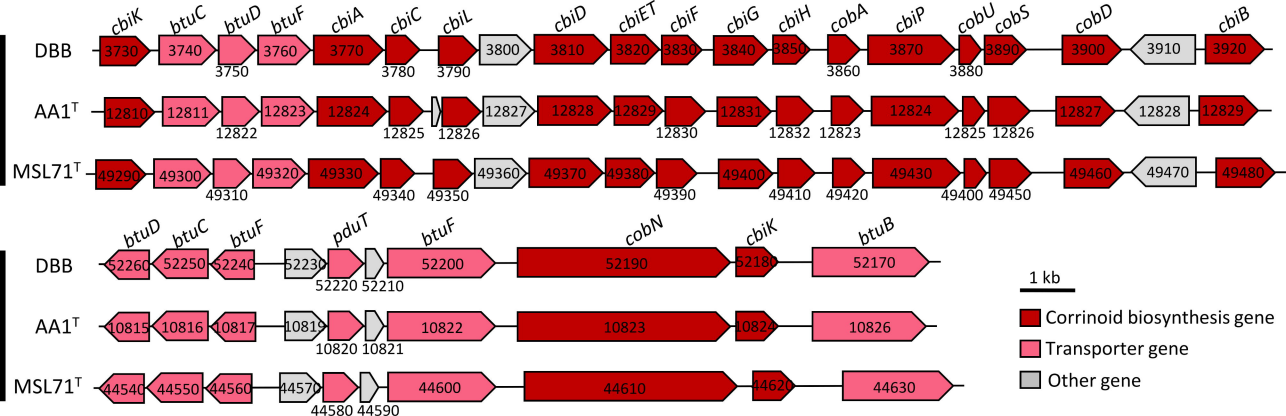
800
600
400
200
0
0
24
48
72
96
120
C
250
200
150
100
50
0
0
4
8
12
16
20
24
B
Relative expression
1000
100
10
1
0.1
0
24
48
72
96
120
Time (hours)
D
1000
100
10
1
0.1
0
4
8
12
24
Time (hours)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btuD
btuF
cbiA
cbiC
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cbiD
cbiET
cbiF
cbiG
cbiH
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cbiP
cobU
cobS
cobD
cbiB
DBB
3730
3740
3750
3760
3770
3780
3790
3800
3810
3820
3830
3840
3850
3860
3870
3880
3890
3900
3910
3920
AA1T
12810
12811
12822
12823
12824
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49340
49350
49360
49370
49380
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49400
49410
49420
49430
49400
49450
49460
49470
49480
btuD
btuC
btuF
pduT
btuF
cobN
cbiK
btuB
DBB
52260
52250
52240
52230
52220
52210
52200
52190
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52170
AA1T
10815
10816
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10819
10820
10821
10822
10823
10824
10826
MSL71T
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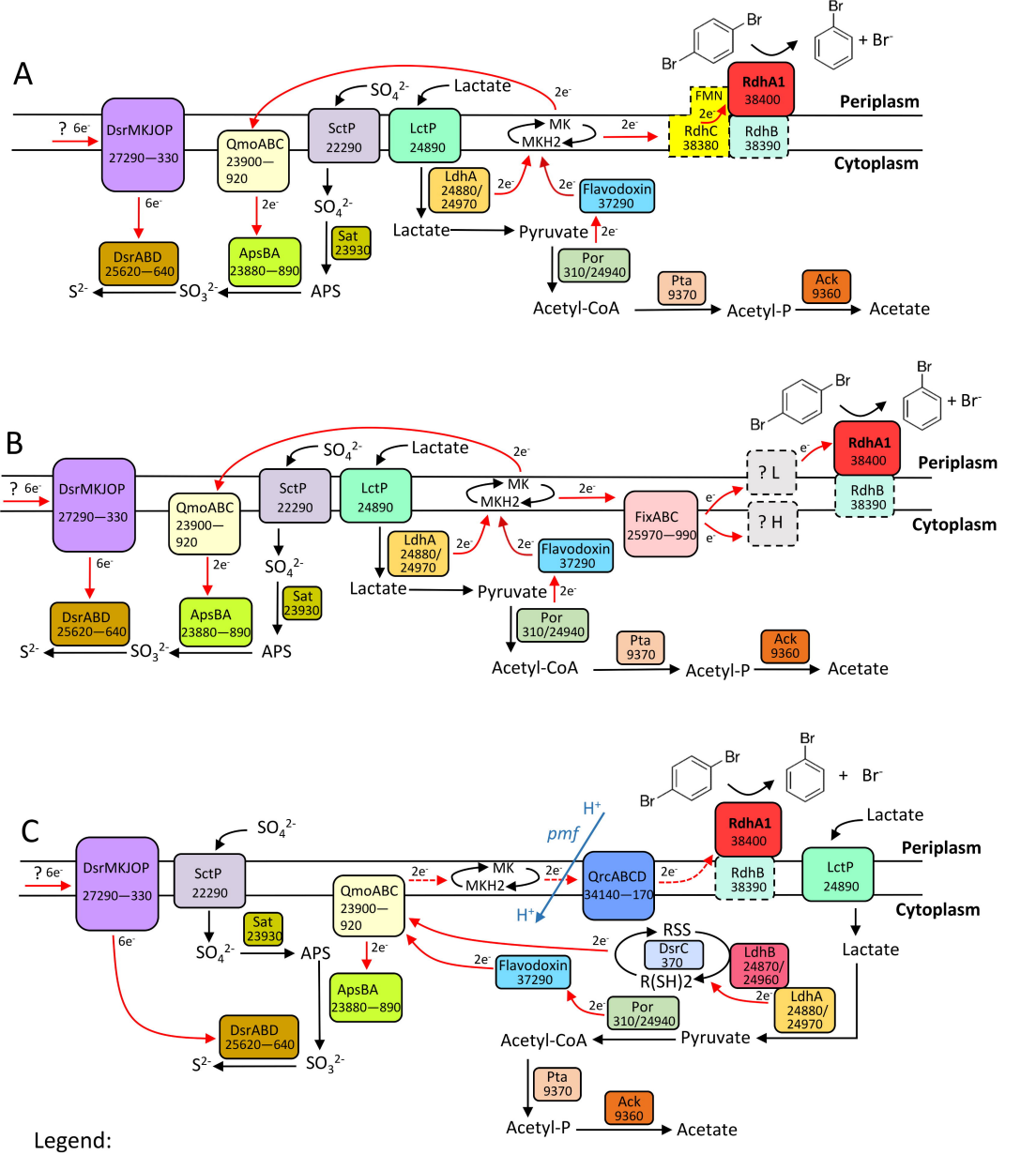
1 kb
Corrinoid biosynthesis gene
Transporter gene
Other gene



Legend:

Ack: acetate kinase
ApsBA: APS reductase
DsrABD: dissimilatory sulfite reductase
DsrC: Dissimilatorysulfitereductase gamma subunit
DsrMKJOP: electron transport complex function with DsrABD
FixABC: electron transfer flavoproteins
H: high potential electron acceptor
L: low potential electron acceptor
LdhA: lactate dehydrogenases
LdhB: HdrD-like putative subunits for LdhA
LctP: lactate permease
MK: menaquinone pool
Por: pyruvate oxidoreductase
Pta: phosphate acetyltransferase
pmf: proton motive force
QmoABC: electron transport complex
QrcABCD: electron transport complex
RdhA1: reductive dehalogenase catalytic subunit
RdhB: putative membrane anchor protein for RdhA1
RdhC: putative electron transport protein for RdhA1
R(SH)2 and RSS, reduced and oxidized forms of thiol-disulfide redox pair
SctP: sulfate permease
Sat: sulfate adenylyltransferase
?: unknown processes or proteins

Table 1. Physiological and genomic properties of *Desulfoluna* strains

Strain	DBB	AA1$^{T\,a}$	MSL71$^{T\,b}$
Isolation source	Marine intertidal sediment	Marine sponge	Estuarine sediment
Cell morphology	Curved rods	Curved rods	Curved rods
Optimum NaCl concentration (%)	2.0	2.5	2.0
Temperature optimum/range (°C)	30/10–30	28/10–36	30/ND^{c}
Utilization of electron donors			
Lactate	+	+	+
Butyrate	+	-	+
Formate	+	+	+
Acetate	-	-	-
Fumarate	-	-	-
Citrate	-	+	-
Glucose	-	+	-
Malate	+	+	+
Pyruvate	+	+	+
Hydrogen	-^{d}	ND	ND
Propionate	-	-	-
Succinate	-	-	-
Utilization of electron acceptors			
Sulfate	+	+	+
Sulfite	+	+	+
Thiosulfate	+	+	+
1,4-Dibromobenzene	+	+^{e}	-^{e}
1,2-Dibromobenzene	-	ND	ND
1,3-Dibromobenzene	-	ND	ND
1,2,4-Tribromobenzene	-	ND	ND
Bromobenzene	-	ND	ND
1,2-Dichlorobenzene	-	ND	ND
1,3-Dichlorobenzene	-	ND	ND
1,4-Dichlorobenzene	-	ND	ND
1,2,4-Trichlorobenzene	-	ND	ND
2-Bromophenol	+	+	+^{e}
4-Bromophenol	+	+	-^{e}
2,4-Dibromophenol	+	+	+$^{e, f}$
2,6-Dibromophenol	+	+	+^{e}
2,4,6-Tribromophenol	+	+	+$^{e, f}$
2-Iodophenol	+	+^{e}	-^{e}
4-Iodophenol	+	+^{e}	-^{e}
2,4-Dichlorophenol	-	-	-^{e}

2,6-Dichlorophenol	-	-	-[e]
2,4,6-Trichlorophenol	-	-	-[e]
Genomic information			
Genome size (Mb)	6.68	6.53 [g]	6.05 [h]
G+C content (%)	57.1	57.9 [g]	57.2 [h]
Total genes	5497	5356 [g]	4894 [h]
Total proteins	5301	5203 [g]	4186 [h]

[a] Data from Ahn et al. (Ahn et al 2009)
[b] Data from Suzuki et al. (Suzuki et al 2008)
[c] ND, not determined
[d] Tested with 1,4-dibromobenzene as the electron acceptor
[e] Data from this study
[f] 4-Bromophenol rather than phenol was the debromination product
[g] Data from GenBank (accession number: NZ_FMUX01000001.1)
[h] Predicted based on draft genome