Proteogenomic Insights into the Physiology of Marine, Sulfate-Reducing, Filamentous *Desulfonema limicola* and *Desulfonema magnum*

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Keywords

Complete genome · Differential proteomics · Metabolism · Anaerobic degradation · Aromatic compounds · Sulfate reduction · Physiology · Stress response · *Desulfonema limicola* · *Desulfonema magnum*

Abstract

The genus *Desulfonema* belongs to the deltaproteobacterial family *Desulfobacteraceae* and comprises marine, sulfate-reducing bacteria that form filaments and move by gliding. This study reports on the complete, manually annotated genomes of *Dn. limicola* 5ac10^T (6.91 Mbp; 6,207 CDS) and *Dn. magnum* 4be13^T (8.03 Mbp; 9,970 CDS), integrated with substrate-specific proteome profiles (8 vs. 11). The richness in mobile genetic elements is shared with other *Desulfobacteraceae* members, corroborating horizontal gene transfer as major driver in shaping the genomes of this family. The catabolic networks of *Dn. limicola* and *Dn. magnum* have the following general characteristics: 98 versus 145 genes assigned (having genomic shares of 1.7 vs. 2.2%), 92.5 versus 89.7% proteomic coverage, and scattered gene clusters for substrate degradation and energy metabolism. The *Dn. magnum* typifying capacity for aromatic compound degradation (e.g., *p*-cresol, 3-phenylpropionate) requires 48 genes organized in operon-like structures (87.7% proteomic coverage; no homologs in *Dn. limicola*). The protein complements for aliphatic

compound degradation, central pathways, and energy metabolism are highly similar between both genomes and were identified to a large extent (69–96%). The differential protein profiles revealed a high degree of substrate-specificity for peripheral reaction sequences (forming central intermediates), agreeing with the high number of sensory/regulatory proteins predicted for both strains. By contrast, central pathways and modules of the energy metabolism were constitutively formed under the tested substrate conditions. In accord with their natural habitats that are subject to fluctuating changes of physicochemical parameters, both *Desulfonema* strains are well equipped to cope with various stress conditions. Next to superoxide dismutase and catalase also desulfoferredoxin and rubredoxin oxidoreductase are formed to counter exposure to molecular oxygen. A variety of proteases and chaperones were detected that function in maintaining cellular homeostasis upon heat or cold shock. Furthermore, glycine betaine/proline betaine transport systems can respond to hyperosmotic stress. Gliding movement probably relies on twitching motility via type-IV pili or adventurous motility. Taken together, this proteogenomic study demonstrates the adaptability of *Dn. limicola* and *Dn. magnum* to its dynamic habitats by means of flexible catabolism and extensive stress response capacities.

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Dedicated to Fritz Widdel who discovered *Desulfonema*.

Introduction

Dissimilatory sulfate-reducing bacteria (SRB) couple the oxidation of organic molecules to the reduction of sulfate (SO_4^{2-}) to sulfide (S^{2-}) and resume diverse functions in ecology, health, and biotechnology [Widdel, 1988; Muyzer and Stams, 2008; Rabus et al., 2015]. The high concentration of sulfate (28 mM) in ocean waters facilitates a central role of SRB in the oxidation of organic matter in anoxic marine sediments and thereby in the global cycles of carbon and sulfur [Jørgensen, 1982; Canfield et al., 1993; Bowles et al., 2014]. These process contributions are mainly performed by the completely oxidizing (to CO_2) and nutritionally versatile members of the deltaproteobacterial family *Desulfobacteraceae* [Fenchel and Jørgensen, 1977; Devereux et al., 1989; Küver, 2014]. To elucidate the molecular basis of this important SRB family, several metabolically/ecologically relevant members have been subjected to genomic and differential proteomic analyses in our group: the facultative chemolithoautotrophic *Desulfobacterium autotrophicum* HRM2 [Strittmatter et al., 2009; Amann et al., 2010; Dörries et al., 2016b], the aromatic compound-degrading *Desulfobacula toluolica* Tol2 [Wöhlbrand et al., 2013, 2016], and the versatile *Desulfococcus multivorans* DSM 2059 [Dörries et al., 2016a].

Morphologically outstanding within the family *Desulfobacteraceae* is the genus *Desulfonema*, which harbors filamentous SRB performing gliding movement. This genus was discovered by Fritz Widdel in the early 1980s by developing specific procedures for enriching and isolating filamentous bacteria [Widdel, 1983]. These new approaches rendered possible the isolation of *Desulfonema limicola* 5a10^T from the mud flat of the Jadebusen (Germany) and *Desulfonema magnum* 4be13^T from anaerobic sediment of a sea water lagoon (Southern France) [Widdel et al., 1983]. By comparison, *Dn. limicola* has somewhat smaller cells and is capable of chemolithoautotrophy, while *Dn. magnum* forms larger cells/longer filaments, utilizes aromatic compounds and accumulates large amounts of poly(3-hydroxybutyrate) during anaerobic growth with benzoate [Widdel et al., 1983; Hai et al., 2004]. *Dn. ishimotonii* Tokyo 01^T was isolated from marine sediment of Tokyo Bay (Japan) and shows nutritional similarity to *Dn. limicola* [Fukui et al., 1999]; its recently determined genome (9 contigs) also revealed extensive impact of horizontal gene transfer [Watanabe et al., 2019]. Phylotypes of *Desulfonema* spp. were detected in microbial mats of geographically distant regions: Solar Lake (Sinai, Egypt) [Teske et al., 1998; Minz et al., 1999], Camar-

gue (France) [Fourçans et al., 2008], Shark Bay (Western Australia) [Wong et al., 2015], and Zodletone spring (OK, USA) [Elshahed et al., 2003]. Furthermore, *Desulfonema* phylotypes have been observed in sheaths of marine, nitrate-reducing, sulphur-oxidizing *Thioploca* spp. on the Chilean continental shelf [Teske et al., 2009], in the seagrass root microbiome from the Leschenault Estuary (Bunbury, Western Australia) [Martin et al., 2020], as well as in rice roots (Vercelli, Italy) [Scheid and Stubner, 2001]. These reports on isolates and phylotypes demonstrate the broad geographic distribution of *Desulfonema* spp. and indicate an adaptation to dynamic gradient systems, including anoxic-oxic transitions.

The aims of the present study were (i) to provide complete, manually annotated genomes of two representatives of the genus *Desulfonema*, namely *Dn. limicola* and *Dn. magnum*, and thus of further members of the family *Desulfobacteraceae*, (ii) to compare their predicted metabolic potentials by integrating differential proteomic profiles of substrate-adapted cells, and (iii) to explore the proteogenomic imprint of their specific lifestyles.

Results and Discussion

General Genome Features

With sizes of 6.91 Mbp and 8.03 Mbp, respectively, the genomes of *Dn. limicola* 5a10^T and *Dn. magnum* 4be13^T, like that of recently reported *Dn. ishimotonii* Tokyo 01^T (6.64 Mbp contig assembly) [Watanabe et al., 2019], are considerably larger than those of other *Desulfobacteraceae* members, ranging between 4.46 Mbp (*Dc. multivorans*) [Dörries et al., 2016a] and 5.59 Mbp (*Dt. autotrophicum*) [Strittmatter et al., 2009], and by far exceed those of incompletely oxidizing (to acetate) members of the family *Desulfovibrionaceae* (e.g., 3.77 Mbp for *Desulfovibrio vulgaris* Hildenborough [Heidelberg et al., 2004]). Figure 1 provides a graphical overview of the circular genomes of *Dn. limicola* and *Dn. magnum*, high-

Fig. 1. Structural representation of the circular chromosomes of *Desulfonema limicola* 5a10^T (top) and *Desulfonema magnum* 4be13^T (bottom). The insert defines the color coding of rings for the selected functions. The scale (Mbp) is indicated by the outer ring. Chemical structures of growth supporting aromatic and aliphatic compounds are presented at the outside. Compound numbering is as follows: 1, 4-hydroxybenzoate; 2, benzoate; 3, *p*-cresol; 4, 3-phenylpropionate; 5, phenylacetate; 6, *n*-propanol; 7, propionate; 8, succinate; 9, fumarate; 10, malate; 11, lactate; 12, butyrate; 13, valerate; 14, acetate; 15, carbon dioxide.

(For figure see next page.)

Table 1. General genome features

Genome features	<i>Desulfonema limicola</i> 5ac10 ^T	<i>Desulfonema magnum</i> 4be13 ^T	<i>Desulfonema ishimotonii</i> Tokyo 01 ^{T a}	<i>Desulfococcus multivorans</i> 1be1 ^b	<i>Desulfobacula toluolica</i> Tol2 ^{T c}	<i>Desulfobacterium autotrophicum</i> HRM2 ^d
Size, bp	6,908,045	8,027,777	6,638,737	4,455,399	5,197,905	5,589,073
Sequencing status	complete	complete	9 contigs	complete	complete	complete
G + C content, mol%	39	45	68	57	42	49
rRNA operons	2	1	4	3	4	6
tRNAs	61	68	76	54	48	50
Coding sequences (CDS)	6,207	9,970	4,997	3,942	4,375	4,943
Coding, %	89.9	86.9	79	87	87	88
Average size, bp	1,001	700	nd	985	1,039	1,017
Assigned function	3,929	4,015	3,724	2,648	3,188	3,477
Conserved unknown	1,173	1,121	1,273 ^e	689	681	1,466
Unknown	1,105	4,834	nd	605	512	nd
Plasmids	–	–	nd	–	–	1
Transposases, IS-elements	703	351	nd	45	163	103
Phage	1	2	nd	–	1	1
CRISPR	8	20	6	6	3	1
Accession number	CP061799	CP061800	BEXT01000000	CP015381	FO203503	CP001087

^a Data taken from Watanabe et al. [2019]. ^b Data taken from Dörries et al. [2016a]. ^c Data taken from Wöhlbrand et al. [2013]. ^d Data taken from Strittmatter et al. [2009]. ^e Without predicted functions. nd, not determined.

lighting the respective genetic repertoires for metabolism and mobilome. General genome features are given and compared to other members of the family *Desulfobacteraceae* in Table 1. Interestingly, although the genomes of *Dn. limicola* and *Dn. ishimotonii* Tokyo 01^T are of rather similar size (differing by 0.25 Mbp), that of *Dn. limicola* possesses more protein-coding sequences (CDS). Furthermore, the GC content of strain Tokyo 01^T is pronouncedly higher (68%) as compared to the other *Desulfonema* species (39 and 45%) and *Desulfobacteraceae* members (42–57%).

A remarkable feature of the large *Dn. magnum* genome is the very high portion of genes (59.7% of all CDS) encoding proteins of (conserved) unknown function that cumulatively account for ~30% of the genome sequence (Fig. 1; Table 1). Likewise, the genome of *Dn. limicola* codes for a high portion of proteins of unknown function (36.7%) as compared to the other *Desulfobacteraceae* members (<32.8%), *Dv. vulgaris* Hildenborough (33.9%), *Geobacter sulfurreducens* PCA (27.3%), or *Escherichia coli* K12 (14.3%). Thus, one may speculate that some of these genes are the basis for so far unknown properties specific to *Desulfonema* spp. Indeed, 319 proteins of unknown function (including paralogs) are homologous in *Dn. limicola* and *Dn. magnum* (applying >50% identity and covering >50% of sequence length).

Furthermore, 280 homologous proteins of unknown function are also encoded in *Dn. ishimotonii* (in total 1,273), while only <169 are present in the other *Desulfobacteraceae* members, supporting this hypothesis. Acquisition and/or evolution of these genes may have been facilitated by the high number of transposases and IS-elements that account for 3.5% of all CDS in *Dn. magnum* (similar to 3.7% in *Db. toluolica* Tol2) and even 11.3% in *Dn. limicola* (only 0.9% in *Dv. vulgaris* Hildenborough and 1.2% in both *G. sulfurreducens* PCA and *E. coli* K12). Some of these mobile genetic elements are constituents of genomic islands and islets (20/6 and 12/15 for *Dn. limicola* and *Dn. magnum*, respectively). Furthermore, the presence of prophages in the chromosome as well as several CRISPR/Cas loci (8 and 20 for *Dn. limicola* and *Dn. magnum*, respectively) indicate severe phage impact on the genomes of both strains [Horvath and Barrangou, 2010]. Overall, the apparent high genome plasticity mediated by the large number of mobile genetic elements and, hence, facilitating horizontal gene transfer [Oliveira et al., 2017], does not only appear as an overarching property among to date determined genomes of *Desulfobacteraceae* members, but may also have been particularly instrumental for expansion of the two studied *Desulfonema* genomes.

To compare the relevance of clusters of orthologous genes (COGs) between the two studied *Desulfonema* strains, the genomic shares (i.e., portion of total coding sequence) captured by the CDS assigned to each COG category were determined (Fig. 2a; online suppl. Table S1; see www.karger.com/doi/10.1159/000513383 for all online suppl. material). Despite rather similar profiles of the relative shares of COG categories (designations are specified in the legend to Fig. 2) in both strains, several differences were noted: in *Dn. limicola*, a higher portion (difference >0.4 percentage points) in genes of transcription (K), replication, recombination and repair (L), signal transduction (T), defense (V), energy production and conversion (C), lipid transport and metabolism (I), and inorganic ion transport and metabolism (P) was observed. By contrast, *Dn. magnum* revealed higher shares in cell wall/membrane/envelope biogenesis (M), post-translational modification, protein turnover and chaperons (O), intracellular trafficking and secretion (U) and most pronounced in genes not assigned to a COG category (NiC).

Proteomic Dataset and Coverage

Differential proteomic analyses of *Dn. limicola* and *Dn. magnum* were based on 3 types of substrate adaptation conditions: (i) shared by both strains (butyrate, propionate, fatty acids mixture, succinate, and fumarate); (ii) specific for *Dn. limicola* (H_2/CO_2 , *n*-propanol, lactate), and (iii) specific for *Dn. magnum* (malate, benzoate, phenylacetate, 3-phenylpropionate, *p*-cresol, and 4-hydroxybenzoate). Biological replicate samples of each growth condition were analyzed with respect to the composition of the soluble as well as the membrane protein-enriched fractions. Overall, 1,133 and 1,413 nonredundant proteins were identified for *Dn. limicola* and *Dn. magnum*, corresponding to a total proteomic coverage of 18.3 and 26.1%, respectively.

Interestingly, while the genomic share of most COG categories was higher in *Dn. limicola* as compared to *Dn. magnum* (15 out of 27, >0.05% points difference, 5 for *Dn. magnum*), the situation is reversed in case of protein formation (Fig. 2a; online suppl. Table S2). Here, only in 4 categories a higher proportion was detected for *Dn. limicola*, while in 14 categories *Dn. magnum* accounted for a larger share. Most pronounced is this difference for genes related to cellular structure (M), coenzyme metabolism (H), secondary metabolites (Q), and proteins not assigned (NiC). Furthermore, for a number of categories (J, K, L, T, C, E, G, H, P, and S), the formed protein complement of *Dn. magnum* exceeds that of *Dn. limicola*, although the

latter disposes of the larger genomic potential. This difference, however, may also be due to metabolism of aromatic compounds by *Dn. magnum* necessitating a suite of other, non-catabolic proteins (e.g., for detoxification) not required under the conditions tested with *Dn. limicola*. Apparently, both strains share a similar minimum with respect to the use of their genomic potential (protein formed vs. predicted only) of at least 6.8 and 8.3% in case of category L. In contrast, the maximum use is smaller in case of *Dn. limicola* (49.2%, category I) than in *Dn. magnum* (61.2%, category C), and *Dn. magnum* apparently in general employs more of its genome encoded proteins (on average 32.4 vs. 26.0% for *Dn. limicola*), being consistent with the above described observations.

To assess similarities between the different growth conditions per studied *Desulfonema* strain, principle component analysis was applied, using the portion of proteins detected of the genomic potential per COG category (Fig. 2b top and center). For both organisms, the different growth conditions are clearly separated, and category C (including the majority of catabolic enzymes) represents the component showing the most pronounced difference between both strains – to a lesser extent also category I for *Dn. magnum* (online suppl. Fig. S1a, b). For *Dn. limicola*, second degree variance is mainly caused by differences in categories E, J, and S. The clear dominance of categories S and NiC in PC2 differences of *Dn. magnum* underscores the importance of proteins of unknown function (S) and with no assignment to any COG category (NiC) for the physiology of this species. Despite the rather similar catabolic routes for fumarate and succinate, the proteomic response of both strains is apparently not only restricted to (small) catabolic adaptations (reflected by small distances in PC1), but also includes other cellular processes yielding separation in PC2. Interestingly, this second component difference appears to be comparable between both strains, since fumarate and succinate data points reveal rather congruent distance patterns, indicating a similar influence as, for example, the redox state of the substrate (i.e., energy yield possible). The clustering of butyrate and propionate in vicinity of aromatic substrates in case of *Dn. magnum* may in part be attributed to their toxic effects that, similar to the solvent properties of the aromatic compounds, lead to a pronounced stress response. The latter possibly involves rearrangement of the fatty acid and phospholipid composition, as previously observed with denitrifying *Aromatoleum aromaticum* EbN1^T [Trautwein et al., 2008; Zink and Rabus, 2010]. This hypothesis finds support by the here revealed importance of COG category I.

When both organisms are included in this analysis, separation of growth conditions is preserved, but a clear affiliation according to the organisms is evident (Fig. 2b bottom), indicating a generally different proteomic response to (in part similar) growth conditions and, hence, an individual fashion of using the respective genomic potential.

Catabolic Network

Based on the complete genomes of *Dn. limicola* and *Dn. magnum* and the respective differential proteomic datasets, the catabolic networks as well as their substrate-dependent regulation were elucidated. These findings are summarized in consecutive Figures 3–5: Figure 3 illustrates the genetic blueprints of both strains underlying individual pathway modules, including the identification status of the encoded proteins. Figure 4 provides an integrated representation of the catabolic networks constructed for both studied strains. Figure 5 displays a heatmap of the substrate-specific protein profiles across the two catabolic networks, visualizing the various degrees of regulatory stringency. Underlying annotation data are compiled in online suppl. Table S2 and corresponding proteomic data for all substrate conditions tested with the two *Desulfonema* strains are presented in detail in online suppl. Table S3. Overall, the catabolic networks of *Dn. limicola* and *Dn. magnum* are composed of 98 and 145 proteins, respectively, which are proteomically covered by 92.5 versus 89.7%, and the coding genes of which have cumulative genomic shares of 1.6 versus 1.5% (Fig. 2c).

(i) Aromatic Compound Degradation

The capacity to utilize aromatic compounds for growth, previously reported to be specific for *Dn. magnum* [Widdel et al., 1983], requires 48 genes (87.7% pro-

teomic coverage) for the degradation of benzoate, phenylacetate, 3-phenylpropionate, *p*-cresol, and 4-hydroxybenzoate (Fig. 4). These genes are mostly clustered in operon-like structures (Fig. 3a) and completely absent from the genome of *Dn. limicola*. The uptake of phenylacetate supposedly occurs via a specifically formed TRAP transporter, similar to *Db. toluolica* Tol2 [Wöhlbrand et al., 2013] and *Dc. multivorans* [Dörries et al., 2016a], while that of the other tested aromatic compounds remains unclear at present. The peripheral conversion of the tested aromatic compounds to the central intermediate benzoyl-CoA (Fig. 4) follows the reaction sequences previously resolved by proteogenomics for *Db. toluolica* Tol2 [Wöhlbrand et al., 2013], *Dc. multivorans* [Dörries et al., 2016a], and *Ar. aromaticum* EbN1^T [Rabus et al., 2014]. For example, *p*-cresol degradation is initiated via addition to fumarate forming 4-hydroxybenzylsuccinate, which is followed by modified β -oxidation yielding benzoyl-CoA and succinyl-CoA. Conversion of phenylacetate to benzoyl-CoA involves α -oxidation as originally discovered in denitrifying *Thauera aromatica* K172^T [Schneider and Fuchs, 1998]. The degradation of the lignin-monomer phenylpropionate to benzoyl-CoA occurs via β -oxidation of the acyl side chain as previously described for *Ar. aromaticum* EbN1^T [Trautwein et al., 2012]. For reductive dearomatization of benzoyl-CoA via the ATP-independent benzoyl-CoA reductases (class II BCR), subsequent ring-cleavage and β -oxidation to acetyl-CoA moieties, the central benzoyl-CoA pathway of strict anaerobes is employed [Boll et al., 2016]. Notably, besides the catabolic proteins, the operons related to degradation of *p*-cresol, 3-phenylpropionate, and benzoate encode electron transfer flavoproteins (ETF) as well as a corresponding ETF:quinone oxidoreductases (Eqo) that are substrate-specifically formed (Fig. 3a, 5; online suppl.

Fig. 2. Proteogenomic datasets for *Dn. limicola* and *Dn. magnum*. **a** Distribution of coding sequences and their proteomic coverage across the clusters of orthologous groups of proteins (COG). COG categories (in alphabetic order): A, RNA processing and modification; B, chromatin structure and dynamics; C, energy production and conservation; D, cell cycle control, cell division, chromosome partitioning; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; G, carbohydrate transport and metabolism; H, coenzyme transport and metabolism; I, lipid transport and metabolism; J, translation, ribosomal structure and biogenesis; K, transcription; L, replication, recombination, repair; M, cell wall/membrane/envelope biogenesis; N, cell motility; NiC, not in COG; O, posttranslational modification, protein turnover, chaperones; P, inorganic ion transport and metabolism; Q, secondary metabolites biosynthesis, transport and catabolism; R, general function prediction only; S, function unknown; T, signal

transduction mechanisms; U, intracellular trafficking, secretion, vesicular transport; V, defense mechanisms; W, extracellular structures; X, mobilome: prophages, transposons; Y, nuclear structure; Z, cytoskeleton. Mapping to *Dn. limicola* versus *Dn. magnum* and protein prediction versus identification is indicated in the insert. Underlying data are compiled in online suppl. Table S1. **b** PCA plots considering the portion of proteins detected of the genomic potential per COG category for *Dn. limicola* (top), *Dn. magnum* (center), and both strains combined (bottom). Abbreviations (in alphabetic order): But, butyrate; Bz, benzoate; *p*-Cre, *p*-cresol; Lac, lactate; FA-Mix, fatty acid mixture; Fum, fumarate; Mal, malate; 4OHBz, 4-hydroxybenzoate; Phac, phenylacetate; 3-Ppp, 3-phenylpropionate; Prop, propionate; PropOH, propanol; Suc, succinate. **c** Proteomic coverage and genomic share across major modules of the catabolic network (see Fig. 3) comparing the two *Desulfonema* strains.

(For figure see next page.)

p-Cresol (3)

Lactate (11)

Dissimilatory sulfate reduction

(ii) DsrMKJOP complex

(v) Ferredoxin:NAD⁺-oxidoreductase, RnfABCDEG

- C**

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(For legend see next page.)

Table S3). Organization of all pathway-required proteins, including transfer of pathway-derived electrons via specific ETF:Eco systems to the menaquinone-pool, was initially described for *Db. toluolica* Tol2 [Wöhlbrand et al., 2013], subsequently also observed in case of *Dt. autotrophicum* HRM2 [Dörries et al., 2016b] as well as *Dc. multivorans* [Dörries et al., 2016a], and biochemically characterized with *Db. toluolica* Tol2 and other (facultative) anaerobes [Vogt et al., 2019]. Hence, such a modular genomic structure may represent another functional building block for the success of energy-limited *Desulfobacteraceae* members in the environment.

The subproteomes of these catabolic modules for aromatic compounds are formed at varying degrees of specificity in *Dn. magnum* (Fig. 5). Firstly, proteins constituting peripheral reaction sequences (yielding benzoyl-CoA) display profiles of high substrate specificity, which is most pronounced for 3-phenylpropionate and *p*-cresol. Secondly, key enzymes of the central benzoyl-CoA pathway, in particular class II BCR, are formed with all tested aromatic growth substrates, but not or only at markedly lower abundance with aliphatic compounds. Thirdly, downstream components of the benzoyl-CoA pathway concerned with β -oxidation are also observed in cells adapted to growth with aliphatic substrates. Taken together, these subproteome profiles are in good agreement with observations from previous studies on *Db. toluolica* Tol2 [Wöhlbrand et al., 2013] and *Dc. multivorans* [Dörries et al., 2016a].

(ii) Aliphatic Compound Degradation

The catabolic module for aliphatic substrates comprises the least number of compound-specific protein constituents. The most apparent difference in the utilization of aliphatic compounds between *Dn. limicola* and *Dn. magnum* is the incapacity of the latter to grow with lactate, an archetypical substrate for SRB. Accordingly, the genome of *Dn. magnum* lacks the genes for a lactate dehydrogenase, which by contrast is specifically formed in *Dn. limicola*.

Fig. 4. Composite catabolic network of *Dn. limicola* and *Dn. magnum*. Assignment of protein constituents to the two *Desulfonema* strains and the state of identification is indicated in the insert. Putative electron flow is indicated by dashed lines. Proteins marked with stars (*) are present as paralogs in the respective organism. Compound numbering and names are as detailed in the legend to Fig. 1. Predicted functions of gene products and underlying proteomic data are compiled in online suppl. Tables S2 and S3.

(iii) Central Degradation Pathways

Classical β -oxidation of fatty acids splitting off acetyl-CoA moieties, the methylmalonyl-CoA pathway converting propionyl-CoA into acetyl-CoA, and the Wood-Ljungdahl pathway terminally oxidizing acetyl-CoA to CO₂ constitute the central degradation pathways shared by *Dn. limicola* (32 genes, 94.6% proteomic coverage) and *Dn. magnum* (40 genes, 91.1% proteomic coverage). In contrast to aromatic compound catabolism, the genes for these central pathways are not organized in pathway-specific modules, but rather randomly scattered across the respective genome, which is reminiscent of the other genome-sequenced *Desulfobacteraceae* members. Only enzymes of the methylmalonyl-CoA pathway have partly clustered genes (e.g., succinate CoA-ligase, malic enzyme, and propionyl-CoA carboxylase) in both *Desulfonema* strains (Fig. 3b). Interestingly, the gene order in the cluster for the methylmalonyl-CoA pathway is different, which is also the case for genes of the Wood-Ljungdahl pathway. The identified protein constituents of all 3 central degradation pathways are essentially constitutively formed across all tested substrate conditions (Fig. 5).

(iv) Chemolithoautotrophy

The ability of *Dn. limicola* to grow chemolithoautotrophically with H₂ and CO₂ as sole sources of energy and carbon, respectively, is based on the presence of hydrogenase (HynA-D) and the reductive Wood-Ljungdahl pathway [Schauder et al., 1989]. Noteworthy, the Hyn hydrogenase is formed under all substrate conditions tested for *Dn. limicola*, but no other types of hydrogenase as previously observed in a proteomic study with *Dt. autotrophicum* HRM2 [Dörries et al., 2016b]. Absence of genes encoding hydrogenase is apparently causative for the inability of *Dn. magnum* to grow with H₂/CO₂.

(v) Energy Metabolism and Redox Complexes

Both *Desulfonema* strains apparently apply sodium-driven symporters to import sulfate for subsequent cytoplasmic sulfate reduction. The latter proceeds according to the conserved reaction sequence for dissimilatory sulfate reduction via Sat, AprAB, and DsrABC, involving membrane protein complexes for pyrophosphate hydrolysis (HppA) and delivery of electrons for APS and sulfite reduction (QmoABC and DsrJKMP). Furthermore, both *Desulfonema* strains possess additional membrane-located, electron-transferring complexes (Rnf1, Rnf2, Tmc, and Qrc) involved in other relevant redox processes (e.g., Rnf in sodium-linked bioenergetics), which were to the

Fig. 5. Heat-map representation of substrate-specific proteome profiles of *Dn. limicola* and *Dn. magnum*. Growth substrates analyzed in both organisms are highlighted in bold. Compound numbering is as in Fig. 1. Scale of meta scores underlying protein identification is shown at the bottom. Absence of paralogue is indicated in gray. Proteins are sorted as indicated by color-coded grouping (left). Abbreviations are as detailed in the legend to Fig. 2.

Table 2. Lifestyle-relevant genomic repertoire^a

Predicted function	Gene(s)		Status ^b	
	<i>Dn. limicola</i> (Dnl)	<i>Dn. magnum</i> (Dnm)	Dnl	Dnm
Cytoskeleton				
Cell division protein (tubulin-like)	Dnl_44430 (<i>ftsZ</i>)	Dnm_000670 (<i>ftsZ</i>)
Min-system	Dnl_46180 (<i>minE</i>)	Dnm_082240 (<i>minE</i>)
	Dnl_46190 (<i>minD</i>)	Dnm_082230 (<i>minD</i>)
	Dnl_46200 (<i>minC</i>)	Dnm_082220 (<i>minC</i>)
	Dnl_44440 (<i>ftsA</i>)	Dnm_000660 (<i>ftsA</i>)
Cell division protein (actin-like)	Dnl_28030	Dnm_078840
Bactofilin domain-containing protein	Dnl_08050	Dnm_093090
Putative polymer-forming cytoskeletal bactofilin				
Cell division				
Cell shape-determining protein (actin-like)	Dnl_08000 (<i>mreB1</i>)	Dnm_093150 (<i>mreB3</i>)
	Dnl_16830 (<i>mreB2</i>)	Dnm_010210 (<i>mreB2</i>)
	Dnl_44740 (<i>mreB3</i>)	Dnm_010190 (<i>mreB1</i>)
		Dnm_087180 (<i>mreB</i> -like)
Rod shape-determining protein C	Dnl_08010 (<i>mreC</i>)	Dnm_093140 (<i>mreC</i>)
Twitching motility				
Prepilin-type cleavage/methylation domain-containing protein (PilA-like) ^c	Dnl_15920	Dnm_080970
Type IV prepilin-like proteins leader peptide-processing enzyme	Dnl_38440 (<i>pilD</i>)	Dnm_006120 (<i>pilD</i>)
General secretion pathway protein E (PilB-like) ^c	Dnl_24430 (<i>gspE2</i>)	Dnm_052530 (<i>gspE</i>)
	Dnl_07850 (<i>gspE1</i>)
Twitching motility protein	Dnl_13080 (<i>pilT1</i>)	Dnm_086960 (<i>pilT2</i>)
	Dnl_13090 (<i>pilT2</i>)	Dnm_086920 (<i>pilT1</i>)
	Dnl_28420 (<i>pilC</i>)	Dnm_083070 (<i>pilC</i>)
Type IV fimbrial assembly protein	Dnl_15850 (<i>pilQ1</i>)	Dnm_028790 (<i>pilQ</i>)
Type IV pilus biogenesis and competence protein	Dnl_60590 (<i>pilQ2</i>)
Adventurous gliding (<i>M. xanthus</i>)				
Mutual gliding-motility protein (intracellular switch)	Dnl_24490 (<i>mglA1</i>)	Dnm_027490 (<i>mglA1</i>)
	Dnl_35750 (<i>mglA2</i>)	Dnm_059280 (<i>mglA2</i>)
Exposure to molecular oxygen				
Superoxide dismutase	Dnl_13760 (<i>sodD</i>)	Dnm_072600
Catalase	Dnl_39890 (<i>katA</i>)	Dnm_031080 (<i>katA</i>)
Desulfoferrodoxin	Dnl_13990 (<i>dfx</i>)	Dnm_085620 (<i>dfx</i>)
Rubrerythrin	Dnl_04120 (<i>rbr</i>)	Dnm_083040 (<i>rbr1</i>)
		Dnm_085640 (<i>rbr2</i>)
Rubredoxin oxidoreductase	Dnl_15040 (<i>rub1</i>)	Dnm_072290 (<i>rub1</i>)
	Dnl_26780 (<i>rub2</i>)	Dnm_087830 (<i>rub2</i>)
	Dnl_21750 (<i>tpx</i>)	Dnm_016000 (<i>tpx</i>)
Thiol peroxidase	Dnl_04600 (<i>trxA1</i>)	Dnm_005330 (<i>trxA2</i>)
Thioredoxin	Dnl_12230 (<i>trxA2</i>)	Dnm_062440 (<i>trxA1</i>)
	Dnl_19500 (<i>trxA3</i>)
	Dnl_41830 (<i>trxA4</i>)
	Dnl_13950 (<i>cydA</i>)	Dnm_085570 (<i>cydA</i>)
Cytochrome <i>bd</i> ubiquinol oxidase, α -subunit	Dnl_13940 (<i>cydB</i>)	Dnm_085560 (<i>cydB</i>)
Cytochrome <i>bd</i> ubiquinol oxidase, β -subunit				
Hyperosmotic stress				
Glycine betaine/proline betaine transport system				
Substrate-binding protein	Dnl_32390	Dnm_029760
Permease protein	Dnl_32400	Dnm_029780
ATP-binding protein	Dnl_32410	Dnm_029850
Substrate-binding protein		Dnm_019300
Permease protein		Dnm_019320
ATP-binding protein		Dnm_019330
Substrate-binding protein		Dnm_029830
Permease protein		Dnm_029840
Transporter, BCCT family ^d	Dnl_48560
	Dnl_57710

Table 2 (continued)

Predicted function	Gene(s)		Status ^b	
	<i>Dn. limicola</i> (Dnl)	<i>Dn. magnum</i> (Dnm)	Dnl	Dnm
Hypoosmotic stress				
Mechanosensitive ion channel	Dnl_31470	Dnm_071850
Heat shock				
Lon protease	Dnl_08790 (<i>lon1</i>) Dnl_38450 (<i>lon2</i>) Dnl_61580 (<i>lon3</i>)	Dnm_037470 (<i>lon2</i>) Dnm_006140 (<i>lon1</i>) Dnm_041110 (<i>lon3</i>)
ATP-dependent protease	Dnl_25780 (<i>hslV</i>)	Dnm_071130 (<i>hslV</i>)
ATP-dependent protease, ATP-binding subunit	Dnl_25770 (<i>hslU</i>)	Dnm_071120 (<i>hslU</i>)
Heat shock protein	Dnl_04010 (<i>dnaJ1</i>) Dnl_50750 (<i>dnaJ2</i>)	Dnm_006650 (<i>dnaJ1</i>) Dnm_062970 (<i>dnaJ2</i>)
Hsp70 cofactor	Dnl_08340 (<i>grpE</i>)	Dnm_066000 (<i>grpE</i>)
Chaperone Hsp70 family	Dnl_08350 (<i>dnaK</i>)	Dnm_066010 (<i>dnaK</i>)
Chaperone protein	Dnl_63920 (<i>clpB2</i>) Dnl_35250 (<i>clpB1</i>)	Dnm_089190 (<i>clpB</i>)
10 kDa chaperonin	Dnl_55910 (<i>groS</i>)	Dnm_088690 (<i>groS</i>)
60 kDa chaperonin	Dnl_55920 (<i>groL</i>)	Dnm_088700 (<i>groL</i>)
RNA polymerase sigma factor RpoH (σ^{32})	Dnl_45140 (<i>rpoH</i>)	Dnm_082600 (<i>rpoH2</i>) Dnm_000170 (<i>rpoH1</i>)
Cold shock				
ATP-dependent Clp protease, ATP-binding subunit	Dnl_39040 (<i>clpA</i>)	Dnm_028050 (<i>clpA</i>)
ATP-dependent Clp protease, adapter protein	Dnl_06180 (<i>clpS1</i>) Dnl_39050 (<i>clpS2</i>)	Dnm_028040 (<i>clpS</i>)
DNA gyrase, subunit A	Dnl_51900 (<i>gyrA</i>)	Dnm_096390 (<i>gyrA</i>)
Ribosome-binding factor	Dnl_08710 (<i>rbfA</i>)	Dnm_094910 (<i>rbfA</i>)
Extracytoplasmic stress response				
RNA polymerase sigma factor, σ^{24} -family	Dnl_43490 (<i>rpoD</i> -like)	Dnm_010650 (<i>rpoD</i>)
Serine protease, do-like	Dnl_18910 (<i>degP</i>)	Dnm_078690 (<i>degP</i>)
Putative transcriptional regulator, ResC family	Dnl_00880	Dnm_084870
Zinc metallopeptidase, M50 family	Dnl_48590	Dnm_090890
	Dnl_10550
ATP-dependent Clp protease, ATP-binding subunit	Dnl_61570 (<i>clpX2</i>)	Dnm_041120 (<i>clpX2</i>)
	Dnl_52820 (<i>clpX1</i>)	Dnm_040500 (<i>clpX1</i>)
ATP-dependent Clp protease, proteolytic subunit	Dnl_61560 (<i>clpP</i>)	Dnm_041130 (<i>clpP</i>)

^a Details of genes and proteomic identification are provided in online supplementary Table S3. ^b Color coding: white, genes absent; light *versus* dark teal (Dnl)/mauve (Dnm) indicate gene predicted only *versus* gene product identified. ^c Assignment to PilA and PilC ambiguous. ^d BCCT, betaine-choline-carnitine transport.

largest part identified in the membrane protein-enriched fractions. In general, the protein constituents of energy metabolism and redox complexes were constitutively formed in both *Desulfonema* strains (~50 genes, >90% proteomic coverage).

Lifestyle-Relevant Properties

Typifying properties of *Desulfonema* spp. are their filamentous morphology (accompanied by gliding movement on surfaces) and their occurrence in environments characterized by physicochemical gradients (e.g., cyanobacterial microbial mats), suggesting the need to sense and respond to fluctuating environmental conditions.

Genes that can be assigned to these lifestyle properties are compiled in Table 2 with underlying genomic predictions as well as respective proteomic profiles detailed in online supplementary Tables S4 and S5.

(i) Cell Division, Cytoskeleton, and Motility

A variety of filament- and pattern-forming proteins are known in prokaryotes that are involved in cell division, determining cell shape and other cell biology functions [e.g., Haeusser and Margolin, 2016; Ramm et al., 2019; Wagstaff and Löwe, 2018]. Filamentous FtsZ (tubulin-like) forms the Z-ring as central part of the divisome and is assisted by FtsA (actin-like). Their spatiotemporal

orientation at the division plain is controlled by the MinCDE system, preventing the Z-ring from assembling at the cell poles. Both *Desulfonema* strains possess the respective genes, and the majority of encoded proteins was detected in *Dn. limicola*, but only FtsZ in *Dn. magnum* (online suppl. Table S5). Furthermore, the genomes of both *Desulfonema* strains contain genes (no product detected) coding for bactofilin-like proteins, which can play various structural and functional roles in the cytoskeleton of bacteria [Kühn et al., 2010].

Formation of the elongated shape of bacterial cells essentially requires the MreB protein (actin-like) that organizes the elongasome, a multienzyme complex for cell wall synthesis. While both *Desulfonema* genomes encode several paralogous MreBs, only one is constitutively formed in each strain: MreB1 (*Dn. limicola*) and MreB3 (*Dn. magnum*), which share 89.1% sequence identity.

Desulfonema spp. were early on reported to migrate on surfaces by gliding movement [Widdel et al., 1983], raising the question about the underlying mechanism of motility. Therefore, the genomes of *Dn. limicola* and *Dn. magnum* were primarily searched for genes required for gliding movement (widespread type-IV pili as well as more special systems from *Flavobacterium*, *Myxococcus xanthus*, and *Mycoplasma*), but also for swimming by flagella as previously reviewed by Jarrell and McBride [2008]. Both investigated *Desulfonema* strains possess genes for twitching motility by means of type-IV pili (*pil* genes) and for adventurous gliding motility (*mglA* genes) known from *M. xanthus*. While most protein constituents of these two systems were detected in *Dn. limicola*, this was only the case for PilC in *Dn. magnum*. By contrast, neither homologs of classical flagella genes (*flg*, *flh*, *fli*, *flk*, or *mot*), genes for lateral movement by adhesins (*sprB*, *gld*) located on the cell surface, nor genes for *Mycoplasma*-type gliding (*p42*, *gli*) could be found in either of the two studied genomes. Noteworthy, a large portion of the proteins assigned to cell division, cytoskeleton, and motility was identified in *Dn. limicola* by the current proteomic analysis, while this was the case for only few with *Dn. magnum*.

Notably, some proteins of unknown function were related to a function in the cell envelope by the eggNOG database (assigned to the COG category M) and detected under all or a large number of studied growth conditions (online suppl. Table S5). For example, Dnl_52570 and Dnm_074620 were abundantly present under all tested conditions and also share 39.1% sequence identity, suggesting a similar function in both organisms. Also the second most abundant of these proteins of unknown func-

tion assigned to COG M in *Dn. magnum* (Dnm_000300) shares similar identity with a *Dn. limicola* homolog (Dnl_50070), further supporting the hypothesis that proteins of so far unknown function could be involved in the characteristic morphology of *Desulfonema* spp. Overall, the differences in the protein complement related to morphology and movement formed by the two species fit well to the microscopic observation originally reported by Widdel et al. [1983].

(ii) Adaptation to Redox Gradient Systems

As noted before, *Desulfonema* phylotypes have repeatedly been detected in cyanobacterial mats, which are characterized by diurnal changes between oxygen saturation during the light period and high sulfide concentrations due to high sulfate reduction rates [Revsbech et al., 1983; Fründ and Cohen, 1992]. This suggests *Desulfonema* spp. to be capable of dealing with toxic oxygen species, despite SRB being generally considered as strict anaerobes. In fact, *Desulfovibrio* spp. have been reported to adopt several strategies for protecting against oxygen stress, including flocculation [Sigalevich et al., 2000], rubrerythrin, and rubredoxin oxidoreductase in addition to classical superoxide dismutase and catalase [Lumppio et al., 2001], as well as oxygen respiration [Cypionka, 2000]. Both studied *Desulfonema* strains have at their disposal the full spectrum of proteins for protection against oxygen stress, comprising superoxide dismutases, catalase, desulfoferrodoxin, rubrerythrin, and thiol peroxidase, all of which could be identified. The two strains possess genes for rubredoxin oxidoreductase, albeit the products of which were not identified under the tested substrate conditions. Furthermore, both *Desulfonema* strains form a cytochrome *bd* ubiquinol oxidase, which one may speculate to participate in oxygen respiration as a means to reduce the pO_2 rather than to gain energy [Cypionka, 2000]. By contrast, both strains lack the genes for dissimilatory reduction of nitrate (to N_2). Taken together, *Dn. limicola* and *Dn. magnum* are well equipped to cope with periods of exposure to molecular oxygen in their natural environment, in particular occurring in microbial mats that perform oxygenic photosynthesis.

(iii) Adaptation to Stress Conditions

Demanding environmental conditions, such as recurring osmotic and temperature stress, are inherent to habitats like microbial mats and intertidal sediments. Desiccation increases osmolarity leading to collapse of the cell turgor, which various bacteria counteract by accumulating organic osmolytes (compatible solutes) preferentially

via active uptake [Kempf and Bremen, 1998; Krämer, 2010; Wood, 2011]. The genomes of both *Desulfonema* strains harbor genes encoding various ABC transporters for the compatible solutes glycine betaine and proline betaine [e.g., Teichman et al., 2018], with several subunits being detected in the current proteomic dataset. *Dn. limicola* in addition possesses genes for two members of the ion-driven transporters of the BCCT-family (betaine, choline, carnitine) [Ziegler et al., 2010], one of which was detected. By contrast, sudden decrease in external solute concentration (e.g., by rain at low tide) causes rapid water influx into the cell and thereby increase in cell turgor (hypoosmotic stress), which bacteria overcome by using mechanosensitive channels [Morbach and Krämer, 2002]. Such a channel is encoded by a single gene in each of the two studied genomes and the respective product was detected in case of *Dn. limicola*. Taken together, *Dn. limicola* and *Dn. magnum* possess the transporter equipment to deal with hyper- as well as hypoosmotic stress.

The natural habitats of *Desulfonema* spp., e.g., microbial mats residing in hot climates [Minz et al., 1999], can furthermore be expected to challenge microorganisms due to thermal stress. At increasing temperatures, bacteria conduct heat shock response to maintain protein-folding homeostasis by rapidly forming various proteases and chaperones, transcriptionally controlled by the sigma factor σ^{32} (RpoH) [Guisbert et al., 2008; Spiess et al., 1999]. Accordingly, the genomes of both studied *Desulfonema* strains encode several proteases (3 paralogs of Lon, HslUV), heat shock proteins (2 paralogs of DnaJ, DnaK), chaperons (ClpP, GroSL), and the specific sigma factor itself (RpoH). Notably, for *Dn. magnum* all 3 predicted Lon proteases were detected, while only 1 was detected in case of *Dn. limicola*. Lon proteases contribute to cellular homeostasis by decomposing abnormal and certain regulatory proteins [Mahmoud and Chien, 2018]. In general, bacterial response to decreasing temperature includes the formation of a variety of specific (cold shock) proteins to maintain viability of cells at low temperatures. Out of the known cold shock-induced proteins from *E. coli* [Phadtare and Inouye, 2008], the genomic repertoires of *Dn. limicola* and *Dn. magnum* include the ribosome-binding factor RbfA, ATP-dependent protease ClpAS and gyrase GyrA. Taken together, both *Desulfonema* strains are well equipped to maintain cellular function upon up- or downshift of ambient temperature.

Occurrence of *Desulfonema* spp. in the sheaths of *Thioploca* spp. [Teske et al., 2009], as well as their gliding movement [Widdel et al., 1983], can be expected to exert physical straining on their cell envelope, potentially leading to mis-

folded outer membrane proteins. This could be counter-vailed by extracytoplasmic (or envelope) stress response (ESR), which has been well studied in model organisms, such as *E. coli*, in particular the response mediated by σ^E (σ^{24} , RpoE) [Bury-Mone et al., 2009; Rowley et al., 2006]. In *E. coli*, the σ^E -regulon comprises a broad range of genes belonging to different functional categories, such as σ -factors, primary metabolism, proteases, or membrane lipid metabolism (see Table 1 in [Rowley et al., 2006]). Potential members of a σ^E -regulon are also predicted and formed in both studied *Desulfonema* strains (online suppl. Tables S6 and S7), as well as σ^E itself and the associated ClpPX complex transforming σ^E into its active transcription-controlling form. Moreover, the periplasmic DegP protein, which controls protein stability and turnover in the periplasm and the expression of which is under control of σ^E [Spiess et al., 1999], is formed by both *Desulfonema* strains. However, the upfront part for the regulation of the σ^E activity, i.e., the membrane-localized proteins ResA (σ^E -binding) and DegS (ResA-cleaving in the presence of misfolded OMPs), are apparently not encoded in the genome of the two studied *Desulfonema* strains. Thus, the ESR in *Desulfonema* spp. has some unclear facets, possibly differing in parts from the mechanism elucidated with *E. coli*.

Conclusions

The present proteogenomic study suggests the success of the two filamentous sulfate reducers *Dn. limicola* and *Dn. magnum* in their highly dynamic habitats (e.g., tidal marine sediments or cyanobacterial mats) to rely on the large genomic arsenal of stress response mechanisms as well as the metabolic flexibility considering the broad range of utilizable substrates. Furthermore, this genomic potential is apparently employed when appropriate (e.g., substrate availability), mediated by a substantial network of regulatory proteins. It seems plausible that the demanding habitat conditions may have promoted lateral acquisition of functionally advantageous genes from other habitat members and, hence, yielded the increased genome size – a hypothesis supported by the large repertoire of mobile genetic elements. While these properties are generally also characteristic for other *Desulfobacteraceae* members, the genomes (and proteomes) of the two studied *Desulfonema* strains harbor a number of conspicuous features that may be related to their unique characteristics as filamentous, gliding SRBs and provide a solid basis for future investigations into their cell biology.

Materials and Methods

Strains, Media, and General Cultivation Conditions

Desulfonema limicola 5ac10^T (DSM 2076) and *Desulfonema magnum* 4be13^T (DSM 2077) were originally isolated by Widdel [1983] with acetate and benzoate, respectively. For the present study, living cultures of both strains were obtained from Fritz Widdel (Bremen, Germany) and subcultured in our laboratory since then. Both strains were cultivated under sulfate-reducing conditions in a defined, sulfide-reduced, and bicarbonate-buffered mineral medium essentially as previously described by Widdel et al. [1983] with modification derived from Rabus et al. [1993]. The defined mineral medium for *Dn. limicola* was composed of 4 g/L Na₂SO₄, 0.2 g/L KH₂PO₄, 0.25 g/L NH₄Cl, 13.5 g/L NaCl, 2.2 g/L MgCl₂ × 6 H₂O, 0.5 g/L KCl, and 0.15 g/L CaCl₂ × 2 H₂O, while that for *Dn. magnum* contained 4 g/L Na₂SO₄, 0.15 g/L KH₂PO₄, 0.2 g/L NH₄Cl, 20 g/L NaCl, 5 g/L MgCl₂ × 6 H₂O, 0.5 g/L KCl, and 1.4 g/L CaCl₂ × 2 H₂O. The media for both *Desulfonema* strains were further supplemented with 1 mL/L each of trace element mixture, selenite/tungstate, vitamins B₁, B₂, and B₁₂, vitamin mixture, 1 M Na₂S, and 30 mL/L NaHCO₃. The medium for *Dn. magnum* was adjusted to pH 7 and that for *Dn. limicola* to pH 7.6. To provide growth-supporting artificial surfaces, 5 mL/L of 48 g/L AlCl₃ × 6 H₂O (*Dn. limicola*) and 95 g/L KAl(SO₄)₂ × 12 H₂O plus 1.6 mL/L of 1 M Na₂CO₃ (*Dn. magnum*) were added to (precipitated in) the medium prior to inoculation as developed by Widdel et al. [1983]. For inoculation of 400-mL cultures, 5% (v/v) preculture of *Dn. limicola* and 2 mL floc suspension of *Dn. magnum* were used. Cultivation was conducted in 500-mL flat glass bottles sealed with butyl rubber stoppers under an anoxic N₂:CO₂ (90:10, v/v) atmosphere at 28°C, lying flat with careful turning once per day. Cultures of both *Desulfonema* strains were adapted to the respective substrate conditions over 5 passages, starting from cultures adapted to anaerobic growth with the fatty acids mixture. For chemolithoautotrophic growth, 400-mL cultures (in 1-L glass bottles) were provided with a H₂:CO₂ (80:20, v/v) atmosphere in the gas headspace and incubated at 100 rpm on a platform shaker. Organic growth substrates were added from sterile stock solutions (final concentrations in mM indicated in parenthesis): lactate (10), propionate (10), butyrate (4), fatty acids mixture [acetate (10), succinate (1), propionate (1), and valerate (0.5)], succinate (10), fumarate (10), malate (10), *n*-propanol (10), phenylacetate (4), 3-phenylpropionate (5), *p*-cresol (3), and 4-hydroxybenzoate (4). Per substrate condition, at least 12 independent cultures were run to provide biological replicates for proteomic profiling. Due to the filamentous, surface-attached growth behavior of both *Desulfonema* strains, growth could not be monitored by measuring the optical density, but was rather determined indirectly on the basis of produced sulfide, by employing the methylene blue formation reaction as previously described [Aeckersberg et al., 1991]. Purity of the cultures was confirmed by microscopic examination (Axiostar; Zeiss AG, Göttingen, Germany). All chemicals were of analytical grade.

Harvesting of both *Desulfonema* strains was conducted at half-maximal sulfide formation, included washing with buffer (100 mM Tris/HCl, 5 mM MgCl₂ × 6 H₂O, adjusted to pH 7), considering the fragility of the filaments, and avoided cooling on ice in-between centrifugation steps. *Dn. limicola* cultures were slowly decanted into centrifuge cups and pelleted at 14,334 g (30 min, 12°C). Following washing and repeated centrifugation, cell suspensions were transferred to 2-mL microreaction tubes and centrifuged (20,817

g, 30 min, 12°C). Then, the supernatant was carefully removed with a pipette yielding the final cell pellets. *Dn. magnum* cultures were placed in upright position for approximately 10–15 min, allowing cell flocs to settle at the bottom of the glass bottles. Then, the supernatant was carefully decanted leaving 20–50 mL in the cultivation vessel. This remaining cell suspension was transferred to one 50-mL reaction tube (with orifice wider than that of the glass bottles), which was subsequently filled up with washing buffer, carefully swung, and incubated in upright position for 10–15 min. This washing procedure was repeated 2 more times. Then, the supernatant was decanted as much as possible and the remaining cell suspension carefully distributed to 2-mL microreaction tubes. After short centrifugation (20,817 g, 2 min, 12°C), the supernatant was discarded, new cell suspension added, and centrifuged anew. This procedure was repeated until cells from a complete 400-mL culture were concentrated. The final cell pellets of *Dn. limicola* and concentrated cells of *Dn. magnum* were immediately shock-frozen in liquid N₂ and stored at –80°C until further analyses.

DNA Sequencing, Assembly, and Annotation

Isolation of genomic DNA was carried out using the Genomic DNA kit (Qiagen, Hildesheim, Germany) according to the manufacturer's instructions. Recombinant plasmid and fosmid shotgun libraries were constructed, and plasmid libraries generated from sonified DNA as previously described [Rabus et al., 2005]. Additionally, a fosmid library was constructed (>40-fold physical coverage) for data finishing and assembly confirmation (Epicentre Technologies, Madison, WI, USA). Templates for sequencing were obtained by insert amplification via PCR or by plasmid isolation. Sequencing was carried out using ABI3730XL capillary systems (ThermoFisher Scientific, Waltham, MA, USA). PHRAP (<http://www.phrap.org/phredphrapconsed.html>) and Consed [Gordon, 2003] were used to assess sequence quality and perform the assembly (>25-fold coverage) with a quality of <1 error in 100,000 bases.

Structural rRNAs and tRNAs were determined using RNAmmer [Lagesen et al., 2007] and tRNAscan-SE [Lowe and Eddy, 1997]. CDS were predicted by the ORF-finding program Glimmer3 [Delcher et al., 1999] and manually revised and curated using Artemis (v.12.0) [Rutherford et al., 2000] and InterPro [Mitchell et al., 2019]. The generated ORF dataset was screened against non-redundant protein databases (SWISSPROT and TrEMBL) [Bairoch and Apweiler, 2000] and the COG database [Galperin et al., 2015]. Genomic islands and islets (<10 kbp) were predicted applying IslandViewer 3 [Dhillon et al., 2015]. The genome was screened for phage-like regions by PHASTER [Arndt et al., 2016], and CRISPR recognition tool [Bland et al., 2007] served in the detection of CRISPR sequences. The eggNOG database [Huerta-Cepas et al., 2019] was consulted for orthology prediction and functional categorization. Gene sequence comparisons between the two *Desulfonema* strains were examined via BioEdit Sequence Alignment Editor [Hall, 1999].

Analysis of genomic and proteomic data was conducted using Matlab version 2020a (MathWorks, Natick, MA, USA).

Sequence Accession Numbers

The genome sequences of *Dn. limicola* 5ac10^T and *Dn. magnum* 4be13^T have been submitted to GenBank under the BioProjects PRJNA660367 and PRJNA660368, respectively, with accession numbers CP061799 and CP061800, respectively.

Proteomics

(i) Analysis of the Membrane Protein-Enriched Fraction

For analyses of the membrane protein-enriched fraction, two biological replicate samples were prepared per growth condition and bacterial strain essentially as described [Košmehl et al., 2013]. In brief, cell pellets were resuspended and disrupted by means of a FastPrep-24 5G bead beater (MP Biomedicals Inc., Irvin, CA, USA): 0.1 mm silica spheres, 4 times with 5 min break on ice, 6 m/s, 10 s each. The extracts were then treated with ice-cold carbonate prior to protein solubilization using SDS. Protein content of the extracts was determined with the RC-DC assay (Bio-Rad GmbH, Munich, Germany) and 10 µg total protein were separated using 12.5% acrylamide mini-gels. Following electrophoresis, gels were stained with Coomassie Brilliant Blue [Neuhoff et al., 1988] and each sample lane cut into 4 slices. Each slice was cut into small pieces (~1 mm²) prior to washing, reduction, alkylation, and tryptic digest [Košmehl et al., 2013]. Generated peptides were separated applying nano liquid chromatography (Ultimate 3000 nano-RSLC System; ThermoFisher Scientific, Germering, Bavaria, Germany) using a 2-cm trap column (C₁₈, 5 µm bead size, 75 µm inner diameter; ThermoFisher Scientific) coupled to a 25-cm analytical column (C₁₈, 2 µm bead size, 75 µm inner diameter; ThermoFisher Scientific) using a 90-min linear gradient [Wöhlbrand et al., 2016]. Eluting peptides were online ionized (CaptiveSpray™ ion source; Bruker Daltonik GmbH, Bremen, Germany) and mass analyzed by an ion trap (amaZon speed ETD; Bruker Daltonik GmbH). Positive ions were analyzed with a capillary current of 1.3 kV and drygas flow of 3 L/min nitrogen at 150°C. Active precursor exclusion was set for 0.2 min and 20 MS/MS spectra per full scan MS acquired. Protein identification was performed by Mascot (version 2.3; Matrix Science Ltd., London, UK) operated via the ProteinScape platform (version 4.2; Bruker Daltonik GmbH). Search settings were as follows: significance threshold $p < 0.05$; mass tolerance MS 0.3 Da, MS/MS 0.4 Da; false discovery rate 1.0% (applying target decoy); 1 missed cleavage site allowed; oxidation M variable modification; carbamidomethyl C fixed modification.

(ii) Shotgun Proteomics

Shotgun protein analysis covering cell disruption, removal of cell debris, reduction, alkylation, and tryptic in-solution digest was performed as described previously using 3 replicate samples per growth condition [Zech et al., 2013]. Obtained peptide mixtures were separated using a 240-min linear gradient [Wöhlbrand et al.,

2017] and protein identification performed as described above using compilation of replicate samples applying the protein extractor implemented in ProteinScape.

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Statement of Ethics

Ethical approval was not required for this type of study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

R.Ra conceived the study; V.S. manually annotated and analyzed both genomes and integrated the proteomic data; V.S. and L.W. performed data analyses and reconstructed the catabolic network; C.H. did the cultivation work; L.W., S.S., and C.H. conducted differential proteomics; R.Re determined the genomes of both *Desulfonema* strains; R.Ra wrote the manuscript with contributions from L.W. and V.S. All authors have agreed to the final version of the manuscript.

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