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1 **The genome of the epsilonproteobacterial chemolithoautotroph**

2 *Sulfurimonas denitrificans*

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40

41 (abstract)

42 **Sulfur-oxidizing epsilonproteobacteria are common in a variety of sulfidogenic**
43 **environments. These autotrophic and mixotrophic sulfur-oxidizing bacteria are**
44 **believed to contribute substantially to the oxidative portion of the global sulfur**
45 **cycle. In order to better understand the ecology and roles of sulfur-oxidizing**
46 **epsilonproteobacteria, in particular the widespread genus *Sulfurimonas*, in**
47 **biogeochemical cycles, the genome of *Sulfurimonas denitrificans* DSM1251 was**
48 **sequenced. This genome has many features, including a larger size (2.2 Mbp), that**
49 **suggest a greater degree of metabolic versatility or responsiveness to the**
50 **environment than most of the other sequenced epsilonproteobacteria. A branched**
51 **electron transport chain is apparent, with genes encoding complexes for the**
52 **oxidation of hydrogen, reduced sulfur compounds, and formate, and the reduction**
53 **of nitrate and oxygen. Genes are present for a complete, autotrophic reductive citric**
54 **acid cycle. Many genes are present that could facilitate growth in the spatially and**
55 **temporally heterogeneous sediment habitat from where *Sulfurimonas denitrificans***
56 **was originally isolated. Many resistance-nodulation-development-family transporter**
57 **genes (11 total) are present, several of which are predicted to encode heavy metal**
58 **efflux transporters. An elaborate arsenal of sensory and regulatory protein-**
59 **encoding genes is in place, as well as genes necessary to prevent and respond to**
60 **oxidative stress.**

61 Epsilonproteobacteria are represented as molecular isolates from a vast array of
62 habitats, including brackish, marine, and subsurface (e.g., (3, 11, 25, 39, 45, 71, 74);
63 reviewed in (6)). Over the last years, quite a few cultured representatives of this group
64 have been obtained from these environments, and currently all cultured members are
65 chemolithoautotrophs or mixotrophs, capable of using sulfur and/or hydrogen as electron
66 donors, while denitrifying and/or growing as microaerophiles (reviewed in (6)). These
67 organisms use the reductive citric acid cycle for carbon fixation (24, 67). Given their
68 abundance, sulfur-oxidizing epsilonproteobacteria, in particular members of the genus
69 *Sulfurimonas*, are believed to be relevant to the function of the global sulfur cycle (6).

70 Genome data from these organisms would be key to metagenome sequencing
71 efforts in habitats where they are abundant, and would also, by comparison to other
72 epsilonproteobacteria, be helpful for determining the traits unique to a free-living,
73 autotrophic lifestyle versus a host-associated, heterotrophic lifestyle. Recently, the
74 genome sequences of *Sulfurovum* sp. NBC37-1 and *Nitratiruptor* sp. SB155-2, two
75 sulfur-oxidizing epsilonproteobacteria from deep-sea hydrothermal vents were published,
76 which revealed that these organisms share many features with their pathogenic (e.g.,
77 *Campylobacter* and *Helicobacter* spp.) epsilonproteobacterial relatives (41). Given the
78 remarkable variety of habitats where sulfur-oxidizing epsilonproteobacteria are found, it
79 was of great interest to also conduct these analyses on non-vent epsilonproteobacteria. To
80 represent the abundant sulfur-oxidizing epsilonproteobacteria present in coastal marine
81 sediments, we chose to sequence and analyze the genome of the sulfur-oxidizing
82 chemolithoautotroph *Sulfurimonas denitrificans* DSM1251. Based on its phenotype, *S.*
83 *denitrificans* was originally named *Thiomicrospira denitrificans* (70). Subsequent

84 sequencing revealed the polyphyletic nature of the *Thiomicrospiras*, with members from
85 both the gammaproteobacteria and epsilonproteobacteria (40). As a result,
86 *Thiomicrospira denitrificans* was eventually removed from the genus *Thiomicrospira* and
87 placed within the genus *Sulfurimonas* (68). In addition to marine sediments, bacteria
88 belonging to this genus have been isolated or detected in a variety of sulfidogenic
89 environments, including deep-sea hydrothermal vents, the oxic-anoxic interface of
90 marine anoxic basins, and oilfield (6, 18, 25, 32), making organisms of this genus
91 globally significant.

92

93

MATERIALS AND METHODS

94

95 **Genome sequencing, annotation, and analysis.** DNA libraries were created and
96 sequenced to approximately 13X depth of coverage at the Production Genomics Facility
97 of the Joint Genome Institute (JGI) using the whole-genome shotgun method as
98 previously described (7, 57). Gaps were closed and base quality problems were
99 addressed by sequencing finishing reads, and PHRED/PHRAP/CONSED were used for
100 assembly (12, 13, 20). Automated and manual annotation were conducted by ORNL
101 similarly to (7, 57). Results were collated and presented via GenDB (37) for manual
102 verification. The prediction of membrane transporters was based on a transporter
103 annotation pipeline that uses several predictive approaches such as BLAST, COG, PFAM
104 and TIGRFAM HMM searches, transmembrane topology prediction algorithms, and
105 takes advantage of a curated database of transporters. Details of this pipeline and
106 database have been published in (50, 51, 57). The main limitation of this approach is the

107 ability to accurately predict precise transporter specificities. Based on both internal and
108 external testing, this methodology is highly successful at identifying putative transporters
109 and predicting approximate substrate specificity. However, making precise substrate
110 predictions, eg., serine transport rather than amino acid transport, is more problematic.
111 The other related limitation is that the approach is dependent on comparison with known
112 experimentally characterized transporters, so completely novel transporters which have
113 never had homologues experimentally characterized will not be predicted by this
114 methodology.

115 To uncover genes involved in oxidative stress, the *S. denitrificans* genome was
116 examined with a series of BLAST queries, using genes known to be involved in oxidative
117 stress response in *H. pylori* (73).

118 **Identification of genes encoding signal transduction and regulatory proteins.**

119 The complement of genes that encode signal transduction and regulatory proteins were
120 compared among *S. denitrificans* DSM1251, *Thiomicrospira crunogena* XCL-2, and
121 *Nitrosococcus oceani* ATCC 19707. To compare signal transduction and regulatory
122 protein genes among these obligate autotroph genomes, genes were identified by
123 querying the predicted gene products to the InterPro (PRINTS, PFAM, TIGRFAM,
124 PRODOM and SMART) and COGs databases (via HMM search – InterPro, or RPSblast
125 – COGs) to identify domains indicative of a role in these processes (e.g., EAL, GGDEF,
126 PAS/PAC). Genes with predicted domains above the trusted cutoff score (InterPro) or an
127 e value less than e^{-5} (COGs) were assigned a product description and classified using a

128 set of rules based on the domain architecture of the protein. The final results were
129 manually verified.

130 **Nucleotide sequence accession number.** The complete sequence of the *S.*
131 *denitrificans* genome is available from GenBank (accession number NC_007575).

132

133

134 **RESULTS AND DISCUSSION**

135

136 **Genome structure.** The *S. denitrificans* DSM1251 genome is one of the largest
137 epsilonproteobacterial genomes yet sequenced, consisting of a single 2.2 Mbp
138 chromosome (Table 1). The coding density and G+C content are similar to the other
139 epsilonproteobacteria (Table 1). Four rRNA operons are present, which, due to their
140 elevated G+C content (~50%) relative to the genome average (34.5%), are visible as
141 positive G+C content anomalies on the genome map (Fig. 1). Three of these operons
142 (*16S-tRNA^{Ala}-23S-5S*) are 100% identical, and are oriented in the same direction, while
143 the fourth (*16S-tRNA^{Ile}-23S-5S*) is in the opposite orientation, and its 5S and 23S genes
144 each have a single nucleotide substitution compared to the others. The free-living *S.*
145 *denitrificans*, *Sulfurovum* sp. NBC37-1, and *Nitratiruptor* sp. SB155-2 have more rRNA
146 operons than those epsilonproteobacteria that are known to be exclusively host-related
147 (Table 1), which is likely a reflection of an adaptation to fluctuating environmental
148 conditions and the necessity for versatility (30, 41, 65).

149 Two large (17,627 bp), identical transposons are apparent as negative G+C
150 content anomalies (30.0%; Fig. 1). Flanked by identical 12 bp inverted repeats, these

151 transposons (*Suden_0690 – 0702*; *Suden_1587 - 1599*) include genes encoding
152 transposases, as well as proteins similar to the TniB (46%) and TniQ (47%) transposase
153 accessory proteins found in mercury resistance-transposons in *Xanthomonas* sp. W17 and
154 other systems (29). These transposons also include genes encoding a type I restriction-
155 modification methyltransferase and restriction enzyme (see Supplemental Materials).
156 Interestingly, one of the copies of this transposon interrupts a flagellin biosynthetic
157 operon, which may explain why, unlike close relatives (25, 68), this strain of *S.*
158 *denitrificans* is nonmotile (70). Other transposase and integrase genes are described in
159 Supplemental Materials.

160

161 **Transporters.** *S. denitrificans* has a modest complement of genes (97 total)
162 predicted to encode transporters. This number is similar to other sequenced heterotrophic
163 epsilonproteobacteria (75-124 genes), which is surprising given that nutrient
164 requirements for *S. denitrificans*, believed to be an obligate autotroph, are simple,
165 compared to the others. This similarity in transporter numbers is due in part to the
166 expansion of a few transporter families in this species compared to other
167 epsilonproteobacteria. Amt-family transporters, are encoded in the *S. denitrificans*
168 (*Suden_0641* and *0643*), *Sulfurovum* sp. NBC37-1 (2 copies), *Nitratiruptor* sp. SB155-2
169 (1 copy) and *Wolinella succinogenes* (1 copy) genome, but not in *Helicobacter pylori*,
170 *Helicobacter hepaticus*, or *Campylobacter jejuni*, and are likely to facilitate ammonium
171 uptake. Perhaps their absence in *Helicobacter* and *Campylobacter* spp. is due to nitrogen
172 requirements for these species being met primarily from exogenous urea and/or amino
173 acids (35, 54, 69). A FNT (formate-nitrite transporter) gene is present in *S. denitrificans*

174 (*Suden_0716*), and absent from other sequenced epsilonproteobacteria. Neither gene
175 context nor sequence comparison clarifies the substrate for this transporter. Also notable
176 is the presence of an abundance of Resistance-Nodulation-Cell Division (RND)
177 Superfamily genes (11 genes; *Suden_0270*; *Suden_0536*; *Suden_0799*; *Suden_0876*;
178 *Suden_0877*; *Suden_0883*; *Suden_1281*; *Suden_1440*; *Suden_1499*; *Suden_1574*;
179 *Suden_1575*; *Suden_2011*), compared to other epsilonproteobacteria, including the two
180 hydrothermal vent species (2-6 genes). Many of these are predicted to encode
181 transporters for metal efflux in *S. denitrificans*. As in *W. succinogenes* and the two
182 hydrothermal vent epsilonproteobacteria, an apparent operon is present that encodes a
183 cytoplasmic arsenate reductase (*Suden_0314*), arsenite permease (*Suden_0313*), and
184 regulatory protein ArsR (*Suden_0315*; (41, 61). Apparently the sediment ecosystem
185 inhabited by *S. denitrificans* requires a similar or perhaps enhanced level of resistance to
186 metals and other toxins, than the digestive tract habitats and hydrothermal vents favored
187 by the other sequenced species.

188

189 **Electron donors.** *S. denitrificans* was originally isolated in a chemostat with thiosulfate
190 as electron-donor and nitrate as electron-acceptor (70). However, prior to this study, the
191 pathways and complexes involved were not identified. Neutrophilic sulfur-oxidizing
192 bacteria use two types of sulfur oxidation pathways: one involving a multienzyme
193 complex catalyzing the complete oxidation of reduced sulfur compounds to sulfate (Sox
194 pathway) (15, 28), and another implementing sulfite and elemental sulfur as important
195 intermediates (27, 47, 59). The genome of *S. denitrificans* reveals that the oxidation of
196 reduced sulfur compounds proceeds via the Sox pathway (Fig. 2). Homologs for genes

197 encoding all components that are required for a fully functional complex in vitro, i.e.,
198 SoxB, SoxXA, SoxYZ, and SoxCD (15), could be identified. As in other obligate
199 sequenced autotrophs (41, 57), the *sox* genes in *S. denitrificans* do not occur in one
200 cluster, as in the model organism *Paracoccus pantotrophus* GB17 (15), but in different
201 parts of the genome. *S. denitrificans* has basically two clusters, one containing *soxXYZAB*
202 (*Suden_260-264*) and another one containing *soxZYCD* (*Suden_2057-2060*). SoxZY are
203 known to interact with both SoxAB and SoxCD and their duplication could possibly
204 indicate differential regulation of these two loci. SoxCD has homologies to sulfite
205 dehydrogenase (SorAB), but has been shown to act as a sulfur dehydrogenase (15). In
206 addition, it has recently been shown that organisms that lack *soxCD*, but do have *soxB*,
207 *soxBA*, and *soxYZ* use the Sox system to oxidize thiosulfate to sulfur, which is stored
208 either inside the cell or excreted (21). However, elemental sulfur formation by *S.*
209 *denitrificans* has not been reported. Recently, sulfur oxidation enzymes were also
210 measured in the closely related bacteria *Sulfurimonas autotrophica* and *Sulfurimonas*
211 *paralvinellae* (67). In this case, sulfite dehydrogenase was detected using an assay that
212 would not be expected to measure such activity were these organisms to use the Sox
213 system (C. G. Friedrich, pers. comm.), indicating that other *Sulfurimonas spp.* might
214 either not use the Sox system or use a modified version of it. In this regard it is
215 interesting to note that the SoxC sequence identities of *S. denitrificans* to sequences of
216 those organisms that have a contiguous *sox* gene set are significantly lower (44%) than
217 when SoxC sequences from organisms in which *sox* genes occur in one cluster are
218 compared among themselves (>63%). Both *soxB* and *soxC* genes exhibit highest
219 similarities with genes from *Sulfurovum sp.* NBC37-1 (41), which suggests that both

220 clusters of *sox* genes are not recent additions to this epsilonproteobacterial lineage. In
221 fact, a phylogenetic analysis based on a large number of SoxB sequences from a variety
222 of sulfur-oxidizing bacteria is even suggestive of an origin of the *sox* system in
223 epsilonproteobacteria (36).

224 Besides the Sox system, *S. denitrificans* also has a gene encoding a
225 sulfide:quinone oxidoreductase (SQR; *Suden_0619*). SQR catalyzes the oxidation of
226 sulfide to elemental sulfur in *Rhodobacter capsulatus* (56), leading to the deposition of
227 sulfur outside the cells. At present its role in *S. denitrificans* is unclear as this species has
228 not been shown to deposit elemental sulfur, though this possibility has not been
229 exhaustively explored with differing cultivation conditions.

230 The genome also provided evidence for the ability to also use H₂ and formate as
231 electron donors (Fig. 2). Based on this information, *S. denitrificans* was successfully
232 cultivated with H₂ as its electron donor and nitrate as the electron acceptor (Sievert and
233 Molyneaux, unpublished data). The *S. denitrificans* genome encodes two Ni-Fe
234 hydrogenase systems: one cytoplasmic, and one membrane-bound hydrogenase complex.
235 The genes encoding the two subunits of the cytoplasmic enzyme (which lack TAT
236 motifs; *Suden_1437-8*) are adjacent to genes encoding the periplasmic hydrogenase (its
237 small subunit has a TAT motif, and a b-type cytochrome subunit would function to
238 anchor it to the membrane and shunt electrons to the quinone pool; *Suden_1434-6*). The
239 small subunit of the cytoplasmic hydrogenase of *S. denitrificans* forms a cluster with
240 sequences from the two deep-sea hydrothermal vent epsilonproteobacteria and *Aquifex*
241 *aeolicus* and is distantly related to H₂-sensing hydrogenases of alphaproteobacteria and
242 cyanobacteria (41). Nakagawa et al. (41) suggest that the cytoplasmic hydrogenase acts

243 as a H₂-sensing hydrogenase in *Sulfurovum* NBC37-1 and *Nitratiruptor* SB155-2.
244 However, an alternative, and in our view more likely, function for the cytoplasmic
245 enzyme as a catalytically active hydrogenase is suggested by the sequence similarity of
246 both subunits to the enzyme from *A. aeolicus*. In *A. aeolicus*, the cytoplasmic
247 hydrogenase can reduce electron acceptors with very negative redox midpoint potentials,
248 and therefore has been suggested to provide electrons with low potential electrons to the
249 reductive citric acid cycle (4), which would circumvent the necessity for reverse electron
250 transport and thus increase its growth efficiency, similar to what has been found in
251 certain Knallgas bacteria using the Calvin cycle for CO₂-fixation. Further experiments are
252 needed to confirm the actual role of the cytoplasmic hydrogenase. Following these
253 hydrogenase genes are several genes encoding hydrogenase-assembly related functions
254 (*Suden_1433-24*).

255 A formate dehydrogenase complex is encoded in an operon similar in gene order to
256 one found in *W. succinogenes* (*Suden_0816-24*). Formate dehydrogenase α subunits
257 contain a selenocysteine residue (26) which is encoded by a stop codon. A putative
258 selenocysteine codon (TGA) followed by a palindromic region was found between two
259 open reading frames (ORFS) that are homologous to the amino and carboxy ends of
260 formate dehydrogenase; accordingly, these ORFS have been combined into a single
261 coding sequence for the α subunit of this enzyme, *Suden_0820*, which includes the
262 molybdopterin-binding and iron-sulfur cluster domains typically found in this subunit, as
263 well as the TAT-pathway signal sequence which would shunt this subunit to the
264 periplasm. The β and γ subunits are encoded by *Suden_0819* and *Suden_0818*,
265 respectively, with the latter having an unprecedented N-terminal addition with two more

266 predicted transmembrane segments (making it six in total). *Suden_0824* encodes a
267 ferredoxin, which may shuttle the electrons from formate oxidation to cellular processes.
268 Formate dehydrogenase maturation is likely facilitated by the products of *Suden_0823*,
269 which encodes a TorD family protein that functions in molybdoprotein formation, and
270 *Suden_0817*, which encodes a FdhD/NarQ family maturation protein. *Suden_0816*
271 encodes a protein belonging to the aminotransferase class V PFAM, as does the Sela
272 protein, which catalyzes a step in selenocysteinyl-tRNA synthesis. Other genes likely to
273 be involved in selenocysteine synthesis are encoded nearby (*Suden_0830*:
274 selenophosphate synthase; *Suden_0831*: L-seryl-tRNA selenium transferase;
275 *Suden_0832*: selenocysteine-specific translation elongation factor SelB). Interestingly,
276 the hydrothermal vent epsilonproteobacteria do not appear to have this complex. Though
277 a homolog to the α subunit of formate dehydrogenase was present in both *Nitratiruptor*
278 SB155-2 and *Sulfurovum* sp. NBC37-1, genes encoding the other subunits were not
279 apparent from BLASTp searches of their genomes. In addition, *S. denitrificans* has a gene
280 encoding the large subunit of a formate dehydrogenase H (*fdhF*, *Suden_1902*), which is
281 most similar to the one in *W. succinogenes* (WS0126). However, its function and
282 substrate are not apparent based on its sequence or genomic context.

283

284 **Electron acceptors.** All genes required for the complete reduction of nitrate to N₂
285 are present (Fig. 2). However, *S. denitrificans* has some notable modifications compared
286 to the canonical denitrification pathway. Similar to *Bradyrhizobium japonicum*, *S.*
287 *denitrificans* appears to have only a periplasmic nitrate reductase (Nap) and not a
288 cytoplasmic membrane-bound nitrate reductase (Nar) (2), which is present in most

289 organisms producing N₂ from nitrate (52). The *nap* gene cluster (*NapABHGFLD*;
290 *Suden_1514-1519, 1521*) has the same arrangement as the one identified in *W.*
291 *succinogenes*, which is unusual in that it lacks a gene encoding the NapC subunit (64).
292 Possibly, *S. denitrificans* is able to denitrify under microaerobic conditions, as the Nap
293 enzyme has been implicated in aerobic denitrification (38). Nitrite reduction to nitric
294 oxide is likely catalyzed by a cytochrome cd₁-dependent nitrite reductase (*nirS, nirF*;
295 *Suden_1985, 1988*), whose genes are present in a gene cluster (*Suden_1976-1989*) that
296 also includes siroheme synthesis genes and two genes annotated as *norCB* (*Suden_1983-*
297 *1984*).

298 The *norCB* genes, which encode the small and large subunits of nitrous oxide-
299 forming nitric oxide reductase (cNOR), a member of the heme-copper oxidase (HCO)
300 superfamily (16), are usually clustered together with additional genes required for
301 enzyme assembly and activation (76). These latter genes, *norD* and *norQ*, are missing
302 from the *S. denitrificans* genome. While there is evidence for functional cNOR in
303 bacteria that lack the *norQ/cbbQ* gene, there are presently no experimental reports that
304 demonstrate functionality of cNOR in bacteria that also lack the *norD* gene. Attempts to
305 test this for *Hydrogenobacter thermophilus* using *Pseudomonas aeruginosa* or
306 *Escherichia coli* as an expression hosts were inconclusive (66). The *S. denitrificans*
307 *norCB* genes are closest related by sequence similarity to the *norCB* genes in the
308 genomes of *H. thermophilus*, *Methylococcus capsulatus* as well as *Sulfurovum* sp.
309 NBC37-1 and *Nitratiruptor* sp. SB155-2, the latter of which are two newly sequenced
310 marine epsilonproteobacteria (41). All of these genomes also lack the *norD* gene.

311 Even though the functionality of cNOR is questionable, *S. denitrificans* is a

312 complete denitrifier and must be able to reduce NO. Attempts to find other inventory
313 implicated in NO reduction were successful and yielded additional candidate systems.
314 One of them, NADH:flavorubredoxin-NOR, also known as the NorVW complex (17,
315 19), was also not complete and thus likely non-functional because a NorW-encoding gene
316 was not identified. Interestingly, the genome encodes NorV in the unusual form of two
317 genes: one encoding a rubredoxin (*Suden_1582*), which is succeeded by a flavodoxin
318 gene (*Suden_1581*). Although both cNOR and NADH:flavorubredoxin-NOR may not
319 have catalytic activity, it is possible that their NO-binding capacity has a function in NO
320 sequestration and detoxification.

321 Interestingly, the *S. denitrificans* genome also encodes a previously unidentified
322 member of the HCO superfamily that is also a candidate for catalyzing nitric oxide
323 reduction. These HCO genes follow a set of pseudogenes normally involved in nitrate
324 reduction (*Suden_0100-0102*). Based on structural modeling and genome analysis it is
325 expected that this new HCO family is a novel non-electrogenic quinone-oxidizing nitric
326 oxide reductase, gNOR (J. Hemp, M. G. Klotz, L. Y. Stein, R. B. Gennis, unpubl. data).
327 The gNOR family, encoded by the *norGHJ* genes (*Suden_0103-0105*), is unique within
328 the HCO superfamily in that it exhibits a novel active-site metal ligation, with one of the
329 three conserved histidine ligands being replaced with an aspartate. This ligation pattern
330 strongly suggests that the active-site metal is an iron. Structural modeling of members of
331 the gNOR family has identified three conserved acidic residues, which form a charged
332 pocket within the active site, a feature shared with the cNOR family (49). Besides *S.*
333 *denitrificans*, gNOR also appears to be present in *Sulfurovum* sp. NBC37-1 and
334 *Persephonella marina* strain EX-H1, whereas it is missing in *Nitratiruptor* sp. SB155-2.

335 Since phylogenetic analysis demonstrates that nitric oxide reductase activity has evolved
336 multiple times independently within the heme-copper superfamily, these shared features
337 between the distantly related gNOR and cNOR families are interesting examples of
338 convergent evolution (J. Hemp, M. G. Klotz, L. Y. Stein, R. B. Gennis, unpubl. data).

339 Nitrous oxide reduction is carried out by nitrous oxide reductase encoded by an
340 unusual *nos* gene cluster (*Suden_1298-1308*) similar to one previously identified in *W.*
341 *succinogenes* (63). As in *Wolinella*, the NosZ in *S. denitrificans* (*Suden_1298*) contains a
342 C-terminal extension of about 200 residues that carries a monoheme cytochrome c
343 binding motif (CXGCH), suggesting it, too, functions as a cytochrome c nitrous oxide
344 reductase (cNOS; Fig. 2). This feature is also shared by NosZ of *Sulfurovum* sp. NBC37-
345 1 and *Nitratiruptor* sp. SB155-2, which form a cluster with NosZ from *S. denitrificans*
346 (Fig. S3), possibly allowing the design of primers to screen for the presence of
347 denitrifying epsilonproteobacteria in the environment. It has been hypothesized that the
348 *nos* gene cluster in *W. succinogenes* codes for proteins involved in an electron transport
349 chain from menaquinol to cytochrome c nitrous oxide reductase (63), and it is likely that
350 the same holds true for *S. denitrificans*, as well as *Sulfurovum* sp. NBC37-1 and
351 *Nitratiruptor* sp. SB155-2. In addition, *S. denitrificans* has an almost identical copy of
352 *nosZ* next to a c553-type monoheme cytochrome c (*Suden_1770, 1769*), but its function
353 is at this point unknown. Interestingly, *S. denitrificans* also has a gene coding for a large
354 subunit of a ferredoxin-nitrite reductase (*nirB*; *Suden_1241*), which could be involved in
355 nitrite assimilation or detoxification (8). However, no gene coding for the small subunit
356 was identified, raising questions about its function.

357 Additional electron acceptors are suggested by this organism's gene complement

358 (Fig. 2). Like some other delta- and epsilonproteobacteria, the *S. denitrificans* genome
359 contains a cluster of four genes (*Suden_0081-0084*) that encode the FixNOQP proteins,
360 which constitute a proton-pumping *cbb₃*-type cytochrome c oxidase., suggesting an
361 ability to use oxygen as a terminal electron acceptor. This is somewhat unexpected, since
362 *S. denitrificans* was originally described as an obligate denitrifier and is quite sensitive to
363 oxygen (S. Sievert, unpubl. data). *Cbb₃*-type cytochrome c oxidase complexes have
364 extremely high affinities for oxygen (48), which might allow this organism to use oxygen
365 as an electron acceptor under extremely low oxygen tensions, or alternatively, to
366 scavenge oxygen to prevent poisoning. Another possibility might be the involvement of
367 *cbb₃*-type cytochrome c oxidase in the catalytic reduction of NO (14). It is interesting,
368 that, in line with other epsilonproteobacteria, the genome does not contain genes
369 encoding FixGHII, which are present in all other bacteria that express a *cbb₃*-type
370 cytochrome c oxidase and are involved in assembly and maturation of the *cbb₃*-type
371 cytochrome c oxidase complex (33, 44). The reasons for the absence of *fixGHII* in
372 epsilonproteobacteria remain unknown, and it needs to be tested experimentally what the
373 actual role of *cbb₃*-type cytochrome c oxidase in *S. denitrificans* is.

374 Additionally, *S. denitrificans*, *Nitratiruptor* sp. SB155-2, and *Sulfurovum* sp.
375 NBC37-1 may be able to use sulfur compounds as terminal electron acceptors. Genes
376 *Suden_0498-0500* encode an Fe/S-protein, a NrfD-related membrane anchor (8 helices),
377 and an unusually long molybdopterin-containing oxidoreductase that contains a twin-
378 arginine translocation pathway signal, respectively; homologs of these three genes are
379 also encoded adjacent to each other in the two hydrothermal vent epsilonproteobacteria.
380 Together these proteins could form a periplasm-facing membrane-bound complex that is

381 most likely involved in the reduction of sulfur compounds like elemental sulfur,
382 polysulfide, thiosulfate or tetrathionate. However, only a limited number of these
383 molybdopterin-containing oxidoreductases have been characterized and it is presently not
384 possible to infer substrate specificity from the primary structure. Further experiments are
385 needed to confirm the substrate used by this complex.

386

387 **Carbon fixation and central carbon metabolism.** Genes encoding the enzymes
388 of the reductive citric acid cycle are apparent (Fig. 3), which is consistent with prior
389 biochemical and genetic analyses of this organism (24). Of particular note are genes
390 encoding the enzymes necessary for the cycle to operate in the reductive direction:
391 pyruvate:acceptor oxidoreductase (*Suden_0096 – 0099*, based on similarity to
392 biochemically characterized orthologs in *H. pylori* (23)), pyruvate carboxylase
393 (*Suden_0622, Suden_1259*, based on biochemically characterized orthologs from *C.*
394 *jejuni* (72), but see below), 2-oxoglutarate:ferredoxin oxidoreductase (*Suden_1052 – 55*,
395 as for pyruvate:acceptor oxidoreductase, based on (23)), and ATP-dependent citrate lyase
396 (*Suden_0570, Suden_0571*)(24). Also noteworthy is the presence of two copies of
397 succinate dehydrogenase/fumarate reductase, one of which has a subunit that would
398 anchor it to the membrane (*Suden_1028 - 1030*), while the other lacks this subunit and
399 may be cytoplasmic (*Suden_0037, Suden_0038*). The membrane-bound form is unusual
400 in that it has a cysteine-rich, type-E membrane anchor. It is similar to SdhABE from *W.*
401 *succinogenes* which has been characterized as a membrane-bound fumarate-reducing
402 complex with subunits SdhAB facing the periplasm. In contrast to *W. succinogenes*, the
403 *S. denitrificans* gene encoding subunit A is about 43 residues shorter and lacks the TAT

404 signal peptide present in its *W. succinogenes* homolog, something that appears to be
405 shared with *Sulfurovum* sp. NBC37-1 and *Nitratiruptor* sp. SB155-2, both of which are
406 also chemolithoautotrophic epsilonproteobacteria using the reductive citric acid cycle for
407 carbon fixation. Thus, it appears that in these organisms the membrane-bound fumarate-
408 reducing complex faces into the cytoplasm. At present the exact function of the two
409 fumarate reductases is unknown, although it is likely that the membrane-bound one, due
410 to its potential for additional energy generation, might be involved in the reductive citric
411 acid cycle for autotrophic carbon fixation. The intriguing possibility that *S. denitrificans*
412 might also be able to carry out fumarate respiration has to await further experimentation.
413 However, *S. denitrificans* does not contain a *frdCAB* operon typical for
414 menaquinol:fumarate reductase sustaining fumarate respiration in other
415 epsilonproteobacteria.

416 The acetyl-CoA and oxaloacetate produced by the reductive citric acid cycle
417 could be funneled to central carbon metabolism: acetyl-CoA could be converted to
418 pyruvate via pyruvate:acceptor oxidoreductase (see above) and oxaloacetate could be
419 used to form phosphoenolpyruvate via phosphoenolpyruvate carboxykinase
420 (*Suden_1696*). Acetyl-CoA could also be directed to fatty acid synthesis (acetyl-CoA
421 carboxylase; *Suden_1174*; *Suden_1608*). Genes are present that suggest an ability to
422 supplement autotrophic growth with acetate assimilation in all three sulfur-oxidizing
423 epsilonproteobacteria. Two possible systems for converting acetate to acetyl-CoA are
424 present: acetyl-CoA ligase (*Suden_1451*), as well as phosphate acetyltransferase
425 (*Suden_0055*) and acetate kinase (*Suden_0056*), and are also present in *Sulfurovum* sp.
426 NBC37-1, while acetate kinase is absent in *Nitratiruptor* sp. SB155-2. Perhaps the two

427 systems have different affinities for acetate, as has been demonstrated in methanogens
428 (60), and are differentially expressed depending on environmental concentrations of this
429 organic acid.

430 In order for *S. denitrificans* to grow autotrophically using the reductive citric acid
431 cycle, there must be a means of carboxylating pyruvate to form oxaloacetate. In some
432 organisms this is accomplished by the tandem activities of phosphoenolpyruvate
433 synthetase and phosphoenolpyruvate carboxylase, while others use pyruvate carboxylase
434 (55). In contrast to the two autotrophic epsilonproteobacteria *Sulfurovum* NBC37-1 and
435 *Nitratiruptor* SB155-2, which have genes encoding phosphoenolpyruvate synthase as
436 well as pyruvate kinase (41), the latter of which is usually involved in ATP synthesis
437 during glycolysis, *S. denitrificans* does not appear to have any genes that might encode an
438 enzyme that could interconvert phosphoenolpyruvate and pyruvate. Instead, it may use
439 pyruvate carboxylase, as genes encoding both the biotin carboxylase subunit
440 (*Suden_0622*) and biotin carboxyl carrier subunit (*Suden_1259*) of this enzyme are
441 present. The biotin carboxyl carrier subunit gene (*Suden_1259*) occurs in an apparent
442 operon with other genes homologous to the subunits of sodium-transporting oxaloacetate
443 decarboxylase (*Suden_1258 – 60*). *Suden_1259*, which encodes the α subunit of this
444 complex, has a high level of sequence similarity with pyruvate carboxylase genes from
445 various *Campylobacter* species (including one from *C. jejuni* which has been
446 biochemically characterized) (72), while the β and γ subunits (*Suden_1258* and *1260*),
447 which are absent from the heterotrophic epsilonproteobacteria, are similar to those found
448 from *Sulfurovum* sp. NBC37-1, *Nitratiruptor* sp. SB155-2, many gammaproteobacteria,
449 many *Chlorobia*, and *Desulfotalea psychrophila*, a deltaproteobacterial sulfate reducer.

450 Heterotrophic organisms that have this complex ferment citrate. After cleaving citrate to
451 acetate and oxaloacetate, they use the oxaloacetate decarboxylase complex to couple the
452 exothermic decarboxylation of this organic acid to the extrusion of sodium ions. For these
453 organisms, the other genes necessary for citrate fermentation (e.g., citrate transporter) are
454 encoded nearby (9). This is not the case in *S. denitrificans*. An alternative function for
455 *Suden_1259* is suggested by phylogenetic analysis, which places it within a clade with
456 the biochemically characterized pyruvate carboxylase from *C. jejuni* (Fig. 4) and separate
457 from biochemically characterized oxaloacetate decarboxylase genes from *Klebsiella*
458 *pneumoniae* and *Vibrio cholerae* (5). Other members of this clade include the genes from
459 the *Chlorobia*, *Sulfurovum* sp. NBC37-1, and *Nitratiruptor* sp. SB155-2, which also use
460 the reductive citric acid cycle for carbon fixation and are not known to ferment citrate. It
461 is possible to operate the oxaloacetate decarboxylase complex as a pyruvate carboxylase
462 by imposing a sodium gradient across the membrane (10). It is tempting to speculate that
463 in the autotrophic epsilonproteobacteria, the *Chlorobia*, and possibly *D. psychrophila*,
464 this complex functions as a pyruvate carboxylase. Interestingly, the sequenced
465 autotrophic epsilonproteobacteria and *Desulfotalea psychrophila* are all marine
466 organisms, and the *Chlorobia* evolved in the marine environment (1). Only five of the ten
467 sequenced *Chlorobia* have the sodium-transporting oxaloacetate decarboxylase/pyruvate
468 carboxylase complex, while the other five, including *C. tepidum*, have the alpha subunit
469 (on which Fig. 4 is based), but not the three-subunit pump. With the exception of *C.*
470 *limicola*, which was isolated from a mineral hot spring, all *Chlorobia* containing the
471 sodium pump have a requirement for sodium. Thus, it is likely that these organisms have
472 found a way to couple pyruvate carboxylation, which is energetically unfavorable, to a

473 sodium gradient, something that was previously proposed, but never shown in an
474 organism (10). Whether the complex encoded by *Suden_1258-1260* functions as a
475 pyruvate carboxylase or an oxaloacetate decarboxylase is a key point begging
476 clarification, which will be nontrivial, given that a genetic system has not been developed
477 in this organism.

478 Carbon fixed by the reductive citric acid cycle can be shunted through
479 gluconeogenesis, as all genes necessary for this pathway are present. The presence of
480 genes encoding citrate synthase (*Suden_2100*) and ATP-dependent (irreversible)
481 phosphofructokinase (*Suden_0549*) are enigmatic as their roles are unclear in this
482 obligate autotroph.

483 Genes are apparent whose products could utilize the carbon skeletons synthesized
484 by central carbon metabolism for ammonia assimilation (see below) and amino acid,
485 nucleotide, fatty acid, and phospholipids synthesis. Cysteine biosynthesis is notable, in
486 that the reduction of sulfate proceeds via adenosine 5'-phosphosulfate (APS) rather than
487 3'-phosphoadenylylsulfate (PAPS) in a pathway that was until recently only known from
488 plants (42). Genes encoding assimilatory sulfate reduction co-occur in an apparent operon
489 (*Suden_0154-0160*). Most likely this operon-like structure is turned on or off depending
490 on whether *S. denitrificans* is inhabiting an environment with a high concentration of
491 reduced inorganic sulfur compounds. Interestingly, sulfate assimilation in *Sulfurovum* sp.
492 NBC37-1 and *Nitratiruptor* SB155-2 appears to proceed via PAPS.

493

494 **Nitrogen assimilation.** The *S. denitrificans* genome contains *nirC* (*focA*) and
495 *nirB* genes encoding the formate-nitrite transporter (*Suden_0716*) and the large subunit of

496 NAD(P)H-dependent ammonia-forming siroheme nitrite reductase (*Suden_1241*),
497 respectively, along with the inventory for siroheme synthesis (*Suden_1977*, *cysG*,
498 siroheme synthase; *Suden_1988*, *cobA-cysG*, uroporphyrinogen III methylase); however,
499 it lacks the *nirD* gene, which encodes the small subunit of siroheme nitrite
500 reductase. Because the genome also lacks *nrfHA* genes, which encode respiratory nitrite
501 ammonification capacity in many delta- and epsilonproteobacteria (62), it appears that
502 *Sulfurimonas* is solely dependent on ammonia uptake from the environment. The genome
503 contains, indeed, two genes encoding different ammonia permeases (noted above) (22,
504 43), one AmtB-like (*Suden_0641*) and one Rhesus factor-like (*Suden_0643*) permease,
505 which are clustered together with the gene encoding nitrogen regulatory protein PII
506 (*glnK*, *Suden_0642*). Whereas AmtB proteins function as ammonia gas uptake channels,
507 the substrate for Rh-like protein channels is still debated and includes ammonia as well as
508 CO₂ (43). The genome contains also all the additional genes needed for 2-oxoglutarate-
509 sensing and regulation of nitrogen assimilation (reviewed in (34)).

510

511 **Chemotaxis and other regulatory and signaling proteins.** Close relatives of *S.*
512 *denitrificans* are motile, while this particular strain is nonmotile, probably due to the
513 interruption of a flagellar biosynthetic operon by a transposon (see Genome Structure,
514 above). Based on the presence of all of the genes necessary to encode the flagellar
515 apparatus, none of which display any evidence of degeneration, an abundant sensory
516 apparatus necessary to detect the presence of chemoattractants or repellants, and
517 communicate this information to the flagellar motor, as well as the sequence identity of
518 this transposon with a duplicate in the genome (see above), it is likely that nonmotility is

519 a recently acquired property. Interestingly, many of the genes encoding the chemotaxis
520 components are in a large cluster with multiple kinases and response regulators (Fig. 5),
521 as in *Nitratiruptor* sp. SB155-2 (41), suggesting interconnectivity between the
522 chemotaxis and other signal transduction systems. Perhaps the original enrichment and
523 isolation procedure for this strain (in a chemostat) might have selected for a non-motile
524 strain.

525 The *S. denitrificans* genome encodes a relative abundance of signaling proteins.
526 Particularly well-represented in these genomes are genes encoding proteins with EAL
527 and GGDEF domains (based on hits to PFAMS, 16 and 38 genes, respectively), which
528 likely function in the synthesis and hydrolysis of the intracellular signaling compound
529 cyclic diguanylate (53). Further, six proteins with PAS/PAC-domains are encoded, which
530 may function as redox sensors (75). The genomic repertoire of signaling and regulatory
531 genes was compared with two other free-living, obligate chemolithoautotrophs for which
532 these data are available (Table 2). Some features are similar to *Thiomicrospira*
533 *crunogena*: both of these species have a relative abundance of signal transduction
534 proteins compared to *Nitrosococcus oceani* (31), which may be a response to more
535 spatially (sediments; *S. denitrificans*) or temporally (hydrothermal vents; *T. crunogena*)
536 heterogeneous habitats, compared to the open ocean (*N. oceani*). Both have a large
537 number of genes encoding methyl-accepting chemotaxis proteins (MCPs; Table 2).
538 Unlike *T. crunogena*, none of the MCPs from *S. denitrificans* are predicted to have
539 PAS/PAC domains that could bind redox-sensitive cofactors (57), but a gene encoding a
540 protein with a PAS/PAC domain is present in the large cluster of chemotaxis genes
541 described above (Fig. 5), suggesting potential communication between sensing cellular or

542 environmental redox conditions and the chemotactic apparatus. Another similarity
543 between *T. crunogena* and *S. denitrificans* is an abundance of genes predicted to be
544 involved with cyclic nucleotide signal transduction, and many of these are predicted to
545 have EAL and/or GGDEF domains, indicating a role for cyclic diguanylate in
546 intracellular signaling in this organism. Many of these predicted proteins also have
547 PAS/PAC domains, as in *T. crunogena* (57).

548 Unique among the three species compared here, *S. denitrificans* has a relative
549 abundance of signal transduction histidine kinases and an expanded complement of
550 winged helix family two component transcriptional regulators (Table 2). Both *T.*
551 *crunogena* and *N. oceani* can use a rather limited variety of electron donors and
552 acceptors, compared to *S. denitrificans*. Perhaps this expansion in histidine kinases and
553 transcriptional regulators coordinate expression of the complexes necessary for the
554 oxidation of multiple electron donors (e.g., H₂, reduced sulfur compounds, formate), and
555 reduction of multiple electron acceptors (O₂ and NO_x).

556

557 **Oxidative stress.** *S. denitrificans* has reasonably elaborate defenses against
558 oxidative stress, on par with what has been observed for pathogenic
559 epsilonproteobacterium *H. pylori* (73). Initially this was considered surprising to us,
560 because this nonpathogenic species does not have to elude the oxidative arsenal of a host
561 immune system, nor, as a microaerophile capable of growth via denitrification, does it
562 grow in the presence of high concentrations of oxygen. However, given the presence of
563 enzymes with labile iron-sulfur clusters with irreplaceable roles in central carbon
564 metabolism in this organism (e.g., pyruvate:acceptor oxidoreductase; *Suden_0096* –

565 0099; 2-oxoglutarate:acceptor oxidoreductase; *Suden_1053 - 1055*), perhaps added
566 defenses are a necessary part of survival.

567 Several genes are present whose products could prevent the buildup of
568 intracellular Fe²⁺ that can spur hydroxyl radical generation via the Fenton reaction. A
569 gene is present that encodes the Fur protein (*Suden_1272*), which regulates iron uptake
570 (14). A gene encoding iron-binding ferritin (*Suden_1760*) is also present.

571 Many enzymes to detoxify reactive oxygen and nitrogen species are encoded in
572 this genome. An iron/manganese superoxide dismutase (*Suden_1129*) is present in this
573 species, but not the other sulfur-oxidizing epsilonproteobacteria (41). This enzyme and
574 ruberythrin (*Suden_0739*) could convert superoxide to hydrogen peroxide, and this
575 superoxide could in turn be dispatched by catalase (*Suden_1323*), peroxiredoxins
576 (*Suden_0132*, *Suden_0630*, *Suden_1778*, *Suden_1803*), or cytochrome c peroxidase
577 (*Suden_0214*; *Suden_1585*). Peroxiredoxins, particularly alkylhydroperoxide reductases,
578 might be specifically targeted towards low levels of H₂O₂ or organic peroxides (58, 73).
579 To dispense with nitric oxide that escapes the periplasmic and membrane-associated
580 respiratory nitrogen reduction complexes, a truncated hemoglobin gene is present
581 (*Suden_0993*), which may convert nitric oxide to nitrate (46). Thioredoxins
582 (*Suden_0342*; *Suden_0501*; *Suden_1867*; *Suden_2099*) and thioredoxin reductase
583 (*Suden_1869*) could funnel electrons to these oxidative stress proteins, as glutathione
584 does not appear to play this role in this organism, since genes encoding glutathione
585 synthetase or gamma-glutamyl-cysteine ligase are absent.

586 Genes are apparent whose products could enable a cell to cope with damage
587 inflicted by any reactive oxygen or nitrogen species (ROS and RNS) that escape cellular

588 defenses. Endonuclease III (*Suden_0516*) and IV (*Suden_1835*) and MutS (*Suden_0755*)
589 could repair oxidative DNA damage. Methionine sulphoxide reductase (*Suden_0012*) and
590 alkylhydroxide reductase (*Suden_1778*) could contend with any methionine residues or
591 lipids that had been oxidized by interaction with ROS or RNS, while nitroreductases
592 (*Suden_0519*; *Suden_1158*) could prevent oxidized cellular nitrogenous and other
593 compounds from generating peroxide.

594

595 **Conclusions.** *S. denitrificans* has several unique features which differentiate it
596 from the other epsilonproteobacteria that have been sequenced to date. It has a larger
597 genome than most of the others, which likely provides the sensory, regulatory, and
598 metabolic versatility necessary for survival in a habitat more heterogeneous than found in
599 a metazoan host. For example, the numerous genes whose products have redox sensory
600 domains likely function to position these cells in the redoxcline to enable them to obtain
601 the electron donors and acceptors needed for growth. Furthermore, these cells are quite
602 versatile with respect to electron donors and acceptors, as the genome data suggest a
603 capability of using donors and acceptors beyond those based on cultivation studies.
604 Although *S. denitrificans* has been isolated from coastal marine sediments, its genome
605 shares many features with two recently described autotrophic deep-sea hydrothermal vent
606 epsilonproteobacteria, including the potential to utilize a variety of redox substrates
607 (hydrogen gas, reduced sulfur compounds, oxygen, and nitrate), its responses to oxidative
608 stress and high metal content, and a genome size intermediate between the two. This
609 suggests that while these habitats appear at first strikingly different, they require similar
610 adaptations on the scale of the microbes. Several additional features, which are absent in

611 their hydrothermal vent relatives, are present in *S. denitrificans* that may be particularly
612 valuable in the sediment habitat. Their formate dehydrogenase complex would enable *S.*
613 *denitrificans* to utilize a major by-product of fermentation that would co-occur with it
614 should sediment organic carbon loads be high. The presence of the additional oxidative
615 stress protein (superoxide dismutase) may enable *S. denitrificans* to cope with diurnal
616 shifts in sediment oxygen concentration, and several additional RND-family efflux
617 pumps relative to hydrothermal vent epsilonproteobacteria suggest that survival in marine
618 sediments requires a degree of versatility and defense against environmental insult
619 beyond what is necessary at moderate temperatures at hydrothermal vents.

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625

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638 **REFERENCES**

- 639 1. **Alexander, B., J. H. Andersen, R. P. Cox, and J. F. Imhoff.** 2002. Phylogeny
640 of green sulfur bacteria on the basis of gene sequences of 16S rRNA and of the
641 Fenna-Matthews-Olson protein. *Arch Microbiol* **178**:131-140.
- 642 2. **Bedmar, E. J., E. F. Robles, and M. J. Delgado.** 2004. The complete
643 denitrification pathway of the symbiotic, nitrogen-fixing bacterium
644 *Bradyrhizobium japonicum*. *Biochem Soc Trans* **33**:141-144.
- 645 3. **Brettar, I., M. Labrenz, S. Flavier, J. Botel, H. Kuosa, R. Christen, and M. G.**
646 **Hofle.** 2006. Identification of a *Thiomicrospira denitrificans*-Like
647 Epsilonproteobacterium as a Catalyst for Autotrophic Denitrification in the
648 Central Baltic Sea. *Appl Environ Microbiol* **72**:1364-1372.
- 649 4. **Brugna-Guiral, M., P. Tron, W. Nitschke, K. O. Stetter, B. Burlat, B.**
650 **Guigliarelli, M. Bruschi, and M. T. Giudici-Ortoni.** 2003. [NiFe]
651 hydrogenases from the hyperthermophilic bacterium *Aquifex aeolicus*: properties,
652 function, and phylogenetics. *Extremophiles* **7**:145-157.
- 653 5. **Buckel, W.** 2001. Sodium ion-translocating decarboxylases. *Biochim Biophys*
654 *Acta* **1505**:15-27.
- 655 6. **Campbell, B. J., A. S. Engel, M. L. Porter, and K. Takai.** 2006. The versatile
656 epsilon-proteobacteria: key players in sulphidic habitats. *Nature Rev Microbiol*
657 **4**:458-468.
- 658 7. **Chain, P., J. Lamerdin, F. Larimer, W. Regala, V. Lao, M. Land, L. Hauser,**
659 **A. Hooper, M. Klotz, J. Norton, L. Sayavedra-Soto, D. Arciero, N. Hommes,**
660 **M. Whittaker, and D. Arp.** 2003. Complete genome sequence of the ammonia-

- 661 oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europaea*. *J*
662 *Bacteriol* **185**:2759-2773.
- 663 8. **Cole, J.** 1996. Nitrate reduction to ammonia by enteric bacteria: redundancy, or a
664 strategy for survival during oxygen starvation? . *FEMS Microbiol Let* **136**:1-11.
- 665 9. **Dimroth, P.** 2004. Molecular basis for bacterial growth on citrate or malonate. *In*
666 A. Bock, R. Curtiss, J. B. Kaper, F. C. Neidhardt, T. Nystrom, K. E. Rudd, and C.
667 L. Squires (ed.), *EcoSal--Escherichia coli and Salmonella: cellular and molecular*
668 *biology*, vol. 2004. ASM Press, Washington, DC.
- 669 10. **Dimroth, P., and W. Hilpert.** 1984. Carboxylation of pyruvate and acetyl
670 coenzyme A by reversal of the Na⁺ pumps oxaloacetate decarboxylase and
671 methylmalonyl-CoA decarboxylase. *Biochem* **23**:5360-5366.
- 672 11. **Engel, A. S., N. Lee, M. L. Porter, L. A. Stren, P. C. Bennett, and M.**
673 **Wagner.** 2003. Filamentous “Epsilonproteobacteria” Dominate Microbial Mats
674 from Sulfidic Cave Springs. *Appl Environ Microbiol* **69**:5503-5511.
- 675 12. **Ewing, B., and P. Green.** 1998. Basecalling of automated sequencer traces using
676 phred. II. Error probabilities. *Genome Res.* **8**:186–194.
- 677 13. **Ewing, B. L., M. Hillier, P. Wendl, and P. Green.** 1998. Basecalling of
678 automated sequencer traces using phred. I. Accuracy assessment. *Genome*
679 *Res.***8**:175–185.
- 680 14. **Fang, F. C.** 2004. Antimicrobial reactive oxygen and nitrogen species: Concepts
681 and controversies. *Nature Microbiol Rev* **2**:820-832.

- 682 15. **Friedrich, C. G., D. Rother, F. Bardischewsky, A. Quentmeier, and J.**
683 **Fischer.** 2001. Oxidation of Reduced Inorganic Sulfur Compounds by Bacteria:
684 Emergence of a Common Mechanism? *Appl. Environ. Microbiol.* **67**:2873-2882.
- 685 16. **Garcia-Horsman, J. A., B. Barquera, J. Rumbley, J. Ma, and R. B. Gennis.**
686 1994. The superfamily of heme-copper respiratory oxidases. *J Bacteriol*
687 **176**:5587-5600.
- 688 17. **Gardiner, A. M., and P. R. Gardiner.** 2002. Flavohemoglobin detoxifies nitric
689 oxide in aerobic, but not anaerobic, *Escherichia coli*. Evidence for a novel
690 inducible anaerobic nitric oxide-scavenging activity. *J Biol Chem* **277**:8166-8171.
- 691 18. **Gevertz, D., A. J. Telang, G. Voordouw, and G. E. Jenneman.** 2000. Isolation
692 and Characterization of Strains CVO and FWKO B, Two Novel Nitrate-
693 Reducing, Sulfide-Oxidizing Bacteria Isolated from Oil Field Brine. *Appl.*
694 *Environ. Microbiol.* **66**:2491-2501.
- 695 19. **Gomes, C. M., A. Giuffre, E. Forte, J. B. Vicente, L. M. Saraiva, M. Brunori,**
696 **and M. Teixeira.** 2002. A Novel Type of Nitric-oxide Reductase - *Escherichia*
697 *coli* flavorubredoxin. *J Biol Chem* **277**:25273-25276.
- 698 20. **Gordon, D., C. Abajian, and P. Green.** 1998. Consed: a graphical tool for
699 sequence finishing. *Genome Res.* **8**:195-202
- 700 21. **Hensen, D., D. Sperling, H. G. Trüper, D. C. Brune, and C. Dahl.** 2006.
701 Thiosulphate oxidation in the phototrophic sulphur bacterium *Allochromatium*
702 *vinosum*. *Mol Microbiol* **62**:794-810.
- 703 22. **Huang, C. H., and J. Peng.** 2005. Evolutionary conservation and diversification
704 of Rh family genes and proteins. *Proc Natl Acad Sci* **102**:15112-17.

- 705 23. **Hughes, N. J., C. L. Clayton, P. A. Chalk, and D. J. Kelly.** 1998. *Helicobacter*
706 *pylori* *porCDAB* and *oorDABC* Genes Encode Distinct Pyruvate:Flavodoxin and
707 2-Oxoglutarate:Acceptor Oxidoreductases Which Mediate Electron Transport to
708 NADP. *J Bacteriol* **180**:1119-1128.
- 709 24. **Hügler, M., C. O. Wirsén, G. Fuchs, C. D. Taylor, and S. M. Sievert.** 2005.
710 Evidence for autotrophic CO₂ fixation via the reductive tricarboxylic acid cycle
711 by members of the epsilon subdivision of proteobacteria. *J Bacteriol* **187**:3020-
712 3027.
- 713 25. **Inagaki, F., K. Takai, H. Kobayashi, K. H. Nealson, and K. Horikoshi.** 2003.
714 *Sulfurimonas autotrophica* gen. nov., sp. nov., a novel sulfur-oxidizing epsilon-
715 proteobacterium isolated from hydrothermal sediments in the Mid-Okinawa
716 Trough. *Int J Syst Evol Microbiol* **53**:1801-1805.
- 717 26. **Jormakka, M., B. Byrne, and S. Iwata.** 2003. Formate dehydrogenase - a
718 versatile enzyme in changing environments. *Curr Opin Struct Biol* **13**:418-423.
- 719 27. **Kappler, U., and C. Dahl.** 2001. Enzymology and molecular biology of
720 prokaryotic sulfite oxidation. *FEMS Microbiol Let* **203**:1-9.
- 721 28. **Kelly, D. P., J. K. Shergill, W. P. Lu, and A. P. Wood.** 1997. Oxidative
722 metabolism of inorganic sulfur compounds by bacteria. *Antonie Van*
723 *Leeuwenhoek Int J Gen Molec Microbiol* **71**:95-107.
- 724 29. **Kholodii, G. Y., S. Z. Mindlin, I. A. Bass, O. V. Yurieva, S. V. Minakhina,**
725 **and V. G. Nikiforov.** 1995. Four genes, two ends, and a *res* region are involved
726 in transposition of Tn5053: a paradigm for a novel family of transposons carrying
727 either a *mer* operon or an integron. *Mol Microbiol* **17**:1189-1200.

- 728 30. **Klappenbach, J. A., J. M. Dunbar, and T. M. Schmidt.** 2000. rRNA operon
729 copy number reflects ecological strategies of bacteria. *Appl Environ Microbiol*
730 **66**:1328-1333.
- 731 31. **Klotz, M. G., D. J. Arp, P. S. G. Chain, A. F. El-Sheikh, L. J. Hauser, N. G.**
732 **Hommel, F. W. Larimer, S. A. Malfatti, J. M. Norton, A. T. Poret-Peterson,**
733 **L. M. Vergez, and B. B. Ward.** 2006. Complete genome sequence of the marine,
734 chemolithoautotrophic, ammonia-oxidizing bacterium *Nitrosococcus oceani*
735 ATCC 19707. *Appl Environ Microbiol* **72**:6299-6315.
- 736 32. **Kodama, Y., and K. Watanabe.** 2003. Isolation and characterization of a sulfur-
737 oxidizing chemolithotroph growing on crude oil under anaerobic conditions. *Appl*
738 *Environ Microbiol* **69**:107-112.
- 739 33. **Kulajta, C., J. O. Thumfart, S. Haid, F. Daldal, and H. G. Koch.** 2006. Multi-
740 step assembly pathway of the cbb3-type cytochrome c oxidase complex. *J Mol*
741 *Biol* **355**:989-1004.
- 742 34. **Leigh, J. A., and J. A. Dodsworth.** 2007. Nitrogen regulation in bacteria and
743 archaea. *Annu Rev Microbiol* **61**:349-377.
- 744 35. **Mehlman, I. J., and A. Romero.** 1982. Improved Growth-Medium for
745 *Campylobacter* Species. *Appl Environ Microbiol* **43**:615-618.
- 746 36. **Meyer, B., J. F. Imhoff, and J. Kuever.** 2007. Molecular analysis of the
747 distribution and phylogeny of the *soxB* gene among sulfur-oxidizing bacteria --
748 evolution of the Sox sulfur oxidation enzyme system. *Environ Microbiol* **9**:2957-
749 2977.

- 750 37. **Meyer, F., A. Goesmann, A. C. McHardy, D. Bartels, T. Bekel, J. Clausen, J.**
751 **Kalinowski, B. Linke, O. Rupp, R. Giegerich, and A. Puhler.** 2003. GenDB--
752 An open source genome annotation system for prokaryotic genomes. *Nucleic*
753 *Acids Res* **31**:2187-2195.
- 754 38. **Moreno-Vivian, C., P. Cabello, M. Martinez-Luque, R. Blasco, and F.**
755 **Castillo.** 1999. Prokaryotic nitrate reduction: Molecular properties and functional
756 distinction among bacterial nitrate reductases. *J Bacteriol* **181**:6573-6584.
- 757 39. **Moyer, C. L., F. C. Dobbs, and D. M. Karl.** 1995. Phylogenetic diversity of the
758 bacterial community from a microbial mat at an active, hydrothermal vent system,
759 Loihi seamount, Hawaii. *Appl Environ Microbiol* **61**:1555-1562.
- 760 40. **Muyzer, G., A. Teske, C.O. Wirsen, H.W. Jannasch.** 1995. Phylogenetic
761 relationships of *Thiomicrospira* species and their identification in deep-sea
762 hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S
763 rDNA fragments. *Arch Microbiol* **164**:165-172.
- 764 41. **Nakagawa, S., Y. Takaki, S. Shimamura, A. Reysenbach, K. Takai, and K.**
765 **Horikoshi.** 2007. Deep-sea vent epsilonproteobacterial genomes provide insights
766 into the emergence of pathogens. *Proc Natl Acad Sci* **104**:12146-12150.
- 767 42. **Neumann, S., A. Wynen, G. Trüper, and C. Dahl.** 2000. Characterization of the
768 *cys* gene locus from *Allochromatium vinosum* indicates an unusual sulfate
769 assimilation pathway. *Mol Biol Reports* **27**:27-33.
- 770 43. **Peng, J., and C. H. Huang.** 2006. Rh proteins vs Amt proteins: an organismal
771 and phylogenetic perspective on CO₂ and NH₃ gas channels. *Transfus Clin Biol*
772 **13**:85-94.

- 773 44. **Pitcher, R. S., and N. J. Watmough.** 2004. The bacterial cytochrome cbb3
774 oxidases. *Biochim Biophys Acta* **1655**:388-399.
- 775 45. **Polz, M. F., and C. M. Cavanaugh.** 1995. Dominance of one bacterial
776 phylotype at a Mid-Atlantic Ridge hydrothermal vent site. *Proc. Natl. Acad. Sci.*
777 *USA* **92**:7232-7236.
- 778 46. **Poole, R. K.** 2005. Nitric oxide and nitrosative stress tolerance in bacteria.
779 *Biochem Soc Trans* **33**:176-180.
- 780 47. **Pott, A. S., and C. Dahl.** 1998. Sirohaem-sulfite reductase and other proteins
781 encoded in the *dsr* locus of *Chromatium vinosum* are involved in the oxidation of
782 intracellular sulfur. *Microbiol* **144**:1881-1894.
- 783 48. **Preisig, O., R. Zufferey, L. Thony-Meyer, C. Appleby, and H. Hennecke.**
784 1996. A high-affinity *cbb3*-type cytochrome oxidase terminates the symbiosis-
785 specific respiratory chain of *Bradyrhizobium japonicum*. *J Bacteriol* **178**:1532-
786 1538.
- 787 49. **Reimann, J., U. Flock, H. Lepp, A. Honigmann, and P. Adelroth.** 2007. A
788 pathway for protons in nitric oxide reductase from *Paracoccus denitrificans*.
789 *Biochim Biophys Acta* **1767**:362-73.
- 790 50. **Ren, Q., K. Chen, and I. T. Paulsen.** 2007. TransportDB: a comprehensive
791 database resource for cytoplasmic membrane transport systems and outer
792 membrane channels. *Nucl Acids Res* **35**:D274-9.
- 793 51. **Ren, Q., K. H. Kang, and I. T. Paulsen.** 2004. TransportDB: a relational
794 database of cellular membrane transport systems. *Nucl Acids Res* **32**:D284-D288.

- 795 52. **Richardson, D. J., and N. J. Watmough.** 1999. Inorganic nitrogen metabolism
796 in bacteria. *Curr Opin Chem Biol* **3**:207-219.
- 797 53. **Romling, U., M. Gomelsky, and M. Y. Galperin.** 2005. C-di-GMP: the dawning
798 of a novel bacterial signalling system. *Molec Microbiol* **57**:629-639.
- 799 54. **Sachs, G., J. A. Kraut, Y. Wen, J. Feng, and D. R. Scott.** 2006. Urea transport
800 in bacteria: Acid acclimation by gastric *Helicobacter* spp. *J Membr Biol* **212**:71-
801 82.
- 802 55. **Sauer, U., and B. J. Eikmanns.** 2005. The PEP-pyruvate-oxaloacetate node as
803 the switch point for carbon flux distribution in bacteria. *FEMS Microbiol Rev*
804 **29**:765-794.
- 805 56. **Schutz, M., I. Maldener, C. Griesbeck, and G. Hauska.** 1999. Sulfide-quinone
806 reductase from *Rhodobacter capsulatus*: Requirement for growth, periplasmic
807 localization, and extension of gene sequence analysis. *J Bacteriol* **181**:6516-6523.
- 808 57. **Scott, K. M., S. M. Sievert, F. N. Abril, L. A. Ball, C. J. Barrett, R. A. Blake,**
809 **A. J. Boller, P. S. Chain, J. A. Clark, C. R. Davis, C. Detter, K. F. Do, K. P.**
810 **Dobrinski, B. I. Faza, K. A. Fitzpatrick, S. K. Freyermuth, T. L. Harmer, L.**
811 **J. Hauser, M. Hügler, C. A. Kerfeld, M. G. Klotz, W. W. Kong, M. Land, A.**
812 **Lapidus, F. W. Larimer, D. L. Longo, S. Lucas, S. A. Malfatti, S. E. Massey,**
813 **D. D. Martin, Z. McCuddin, F. Meyer, J. L. Moore, L. H. Ocampo, J. H.**
814 **Paul, I. T. Paulsen, D. K. Reep, Q. Ren, R. L. Ross, P. Y. Sato, P. Thomas, L.**
815 **E. Tinkham, and G. T. Zeruth.** 2006. The genome of deep-sea vent
816 chemolithoautotroph *Thiomicrospira crunogena*. *PLoS Biology* **4**:1-17.

- 817 58. **Seaver, L. C., and J. A. Imlay.** 2001. Alkyl hydroperoxide reductase is the
818 primary scavenger of endogenous hydrogen peroxide in *Escherichia coli*. J
819 Bacteriol **183**:7173-7181.
- 820 59. **Shahak, Y., M. Schütz, M. Bronstein, G. Hauska, and E. Padan.** 1999.
821 Sulfide-dependent anoxygenic photosynthesis in prokaryotes: sulfide:quinone
822 reductase (SQR), the initial step, p. 211-228. In G. A. Peshek, W. Löffelhardt, and
823 C. Schmetterer (ed.), The phototrophic prokaryotes. Kluwer Academic/Plenum
824 Publishers, New York.
- 825 60. **Shieh, J. S., and W. B. Whitman.** 1987. Pathway of acetate assimilation in
826 autotrophic and heterotrophic methanococci. J. Bacteriol. **169**:5327-5329.
- 827 61. **Silver, S., and L. T. Phung.** 2005. Genes and enzymes involved in bacterial
828 oxidation and reduction of inorganic arsenic. Appl Environ Microbiol **71**:599-
829 608.
- 830 62. **Simon, J.** 2002. Enzymology and bioenergetics of respiratory nitrite
831 ammonification. FEMS Microbiol Rev **26**.
- 832 63. **Simon, J., O. Einsle, P. H. Kroneck, and W. G. Zumft.** 2004. The
833 unprecedented nos gene cluster of *Wolinella succinogenes* encodes a novel
834 respiratory electron transfer pathway to cytochrome c nitrous oxide reductase.
835 FEBS Letters **569**:7-12.
- 836 64. **Simon, J., M. Sängler, C. Schuster, and R. Gross.** 2003. Electron transport to
837 periplasmic nitrate reductase (NapA) of *Wolinella succinogenes* is independent of
838 a NapC protein. Molec Microbiol **49**:69-79.

- 839 65. **Stevenson, B. S., and T. M. Schmidt.** 2004. Life history implications of rRNA
840 gene copy number in *Escherichia coli*. *Appl Environ Microbiol* **70**:6670-6677.
- 841 66. **Suzuki, M., H. Arai, M. Ishii, and Y. Igarashi.** 2006. Gene structure and
842 expression profile of cytochrome bc nitric oxide reductase from *Hydrogenobacter*
843 *thermophilus* TK-6. *Biosci Biotech Biochem* **70**:1666-1671.
- 844 67. **Takai, K., B. J. Campbell, S. C. Cary, M. Suzuki, H. Oida, T. Nunoura, H.**
845 **Hirayama, S. Nakagawa, Y. Suzuki, F. Inagaki, and K. Horikoshi.** 2005.
846 Enzymatic and genetic characterization of carbon and energy metabolisms by
847 deep-sea hydrothermal chemolithoautotrophic isolated of *Epsilonproteobacteria*.
848 *Appl Environ Microbiol* **71**:7310-7320.
- 849 68. **Takai, K., M. Suzuki, S. Nakagawa, M. Miyazaki, Y. Suzuki, F. Inagaki, and**
850 **K. Horikoshi.** 2006. *Sulfurimonas paralvinellae* sp nov., a novel mesophilic,
851 hydrogen- and sulfur-oxidizing chemolithoautotroph within the Epsilonproteo-
852 bacteria isolated from a deep-sea hydrothermal vent polychaete nest,
853 reclassification of *Thiomicrospira denitrificans* as *Sulfurimonas denitrificans*
854 comb. nov and emended description of the genus *Sulfurimonas*. *Int J Syst Evol*
855 *Microbiol* **56**:1725-1733.
- 856 69. **Testerman, T. L., P. B. Conn, H. L. T. Mobley, and D. J. McGee.** 2006.
857 Nutritional requirements and antibiotic resistance patterns of *Helicobacter* species
858 in chemically defined media. *J Clin Microbiol* **44**:1650-1658.
- 859 70. **Timmer-Ten Hoor, A.** 1975. A new type of thiosulphate oxidizing, nitrate
860 reducing microorganism: *Thiomicrospira denitrificans* sp. nov. . *Netherland J Sea*
861 *Res* **9**:344-350.

- 862 71. **Urakawa, H., N. Dubilier, Y. Fujiwara, D. Cunningham, S. Kojima, and D.**
863 **Stahl.** 2005. Hydrothermal vent gastropods from the same family (Provannidae)
864 harbor gamma- and epsilon-proteobacterial endosymbionts. *Environ Microbiol*
865 **7:750-754.**
- 866 72. **Velayudhan, J., and D. J. Kelly.** 2002. Analysis of gluconeogenic and
867 anaplerotic enzymes in *Campylobacter jejuni*: an essential role for
868 phosphoenolpyruvate carboxykinase. *Microbiol* **148:685-694.**
- 869 73. **Wang, G., P. Alamuri, and R. J. Maier.** 2006. The diverse antioxidant systems
870 of *Helicobacter pylori*. *Molec Microbiol* **61:847-860.**
- 871 74. **Watanabe, K., K. Watanabe, Y. Kodama, K. Sytsubo, and S. Harayama.**
872 2000. Molecular characterization of bacterial populations in petroleum-
873 contaminated groundwater discharged from underground crude oil storage
874 cavities. . *Appl Environ Microbiol* **66: 4803-4809.**
- 875 75. **Zhulin, I., B. Taylor, and R. Dixon.** 1997. PAS domain S-boxes in Archaea,
876 bacteria and sensors for oxygen and redox. *Tr Biochem Sci* **22:331-333.**
- 877 76. **Zumft, W. G.** 2005. Nitric oxide reductases of prokaryotes with emphasis on the
878 respiratory, heme-copper oxidase types. *J Inorg Biochem* **99:194-215.**
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FIGURE LEGENDS

882

883 FIG. 1. Map of the *Sulfurimonas denitrificans* DSM1251 genome. The two outer
884 rings include protein-encoding genes, which are color-coded based on their membership
885 in COG categories. Ring 3 depicts the deviation from the average G+C (%), while the
886 innermost ring is the GC skew ($=\frac{[G-C]}{[G+C]}$). R1, R2, R3, and R4 are ribosomal RNA
887 operons (with their orientation indicated with arrows), and the two regions marked T are
888 identical large transposons. The G+C and GC skew rings were calculated with a sliding
889 window of 10,000 bp with a window step of 100.

890

891

892 FIG. 2. Model for electron transport in *Sulfurimonas denitrificans*. Abbreviations:
893 I—NADH dehydrogenase; II—succinate dehydrogenase/fumarate reductase; III—bc₁
894 complex; IV—cbb₃ cytochrome c oxidase; Cyt_n c—cytochrome c; CCP—cytochrome c
895 peroxidase; FDH—formate dehydrogenase; HYD—hydrogenase; MK—menaquinone;
896 MKH₂—menaquinol; Mo—molybdodenum containing cofactor; NAP—periplasmic
897 nitrate reductase; NIR—nitrite reductase; NOR—nitric oxide reductase; NOS—nitrous
898 oxide reductase; PM—plasma membrane; PSR—polysulfide reductase; SOX—sulfur
899 oxidation system. Candidate monoheme cytochromes c listed as “Cyt_x c” are
900 *Suden_0904*, *Suden_0741*, *Suden_0578* (all COG2863) as well as *Suden_0865*,
901 *Suden_1329* and *Suden_1112* (no COG assignment).

902

903

904 FIG. 3. Central carbon metabolism in *Sulfurimonas denitrificans*. Abbreviations:

905 2-OG—2-oxoglutarate; acCoA—acetyl-Coenzyme A; ACL—ATP-citrate lyase;

906 APFK—ATP-dependent phosphofructokinase; CS—citrate synthase; F6P—fructose 6-

907 phosphate; FBP—fructose 1,6-bisphosphate; FBPP—fructose 1,6 bisphosphate

908 phosphatase; FR—fumarate reductase; fum—fumarate; isocit—iscitrate; mal—malate;

909 MQ—menaquinone; OAA—oxaloacetate; PC—pyruvate carboxylase; PEP—

910 phosphoenolpyruvate; pyr—pyruvate; suc—succinate; suCoA—succinyl-Coenzyme A.

911

912 FIG. 4. Phylogenetic relationships of alpha-subunits of oxaloacetate

913 decarboxylase (OAD), pyruvate carboxylase (PVC), oxoglutarate carboxylase (OGC),

914 and type III pyruvate carboxylase to the product of *Suden_1259* of *Sulfurimonas*

915 *denitrificans*. Sequences were aligned using the program package MacVector. Neighbor-

916 joining and Parsimony trees based on the predicted amino acid sequences were calculated

917 using PAUP 4.0b10. Bootstrap values (1,000 replicates) for the major nodes are given for

918 the neighbor-joining (first value) and parsimony analyses (second value).

919

920 FIG. 5. A large gene cluster from the *Sulfurimonas denitrificans* genome that

921 includes many of the genes for chemotaxis signal transduction.

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TABLE 1. Comparative genome features for epsilonproteobacteria[‡]

929

Species	Size (Mbp)	% coding	%GC	rRNA operons	# CDS
<i>Sulfurimonas denitrificans</i> DSM1251	2.20	93.8	34.5	4	2104
<i>Sulfurovum</i> sp. NBC37-1	2.56	90.1	43.8	3	2466
<i>Nitratiruptor</i> sp. SB155-2	1.88	95.1	39.7	3	1857
<i>Campylobacter fetus</i> 82-40	1.80	90.0	33.3	3	1719
<i>Campylobacter jejuni</i> NCTC 11168	1.64	95.4	30.6	3	1629
<i>Campylobacter jejuni</i> RM1221	1.78	91.8	30.3	3	1838
<i>Helicobacter hepaticus</i> ATCC 51449	1.80	93.4	35.9	1	1875
<i>Helicobacter acinonychis</i> Sheeba	1.55	89.0	38.2	2	1618
<i>Helicobacter pylori</i> 26695	1.67	90.0	38.9	2*	1576
<i>Helicobacter pylori</i> J99	1.64	90.7	39.2	2*	1491
<i>Helicobacter pylori</i> HPAG1	1.59	91.0	39.1	2*	1544
<i>Wolinella succinogenes</i> DSM1740	2.11	94.5	48.5	3	2043

930

931

[‡]Data for all taxa, except for *Sulfurivom* sp. NBC37-1 and *Nitratiruptor* sp.

932

SB155-2 were collated from the Integrated Microbial Genomes webpage and had been

933

generated using consistent methodology. For *Sulfurivom* sp. NBC37-1 and *Nitratiruptor*

934 sp. SB155-2, data were collected from (41), for which slightly different methodologies
935 were used to identify coding sequences (CDS).

936 *In *H. pylori*, the 16S gene is not collocated with the 23S and 5S genes in an
937 operon. Additionally, an orphan 5S sequence is found in strain 26695

938

939

940 TABLE 2. Regulatory and signaling proteins of *Sulfurimonas denitrificans* and other

941 obligate chemolithoautotrophs*

942

Number:			
<i>S. denitrificans</i>	<i>T. crunogena</i>	<i>N. oceani</i>	Functional Description
56	72	104	Transcription/Elongation/Termination Factors
19	4	2	Two component transcriptional regulator, winged helix family
146	128	75	Signal Transduction proteins
28	27	8	Chemotaxis Signal Transduction proteins
13	14	1	Methyl-accepting chemotaxis proteins
118	101	67	Non-Chemotaxis Signal Transduction
36	17	18	Signal Transduction Histidine Kinase
42	49	16	Cyclic nucleotide signal transduction
202	200	179	Total

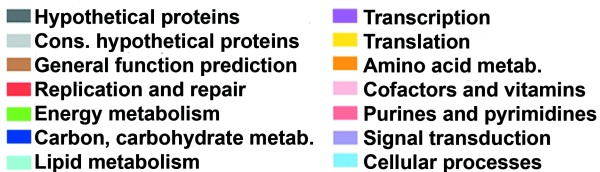
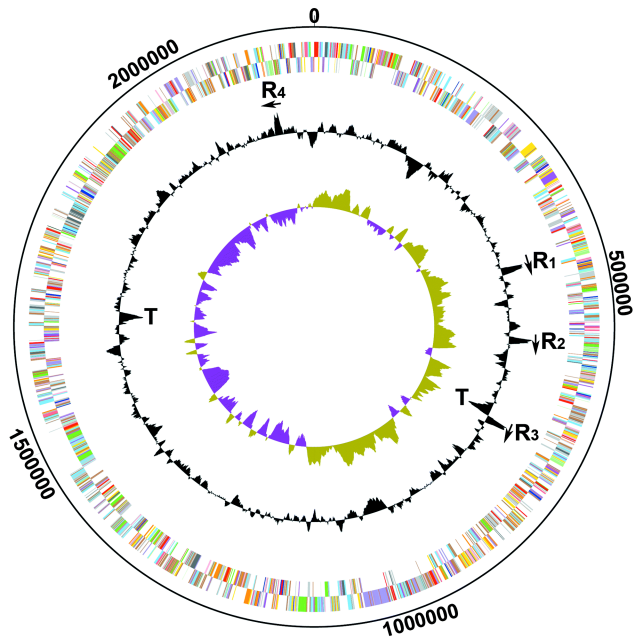
943 **Sulfurimonas denitrificans* DSM1251 is compared to gammaproteobacteria

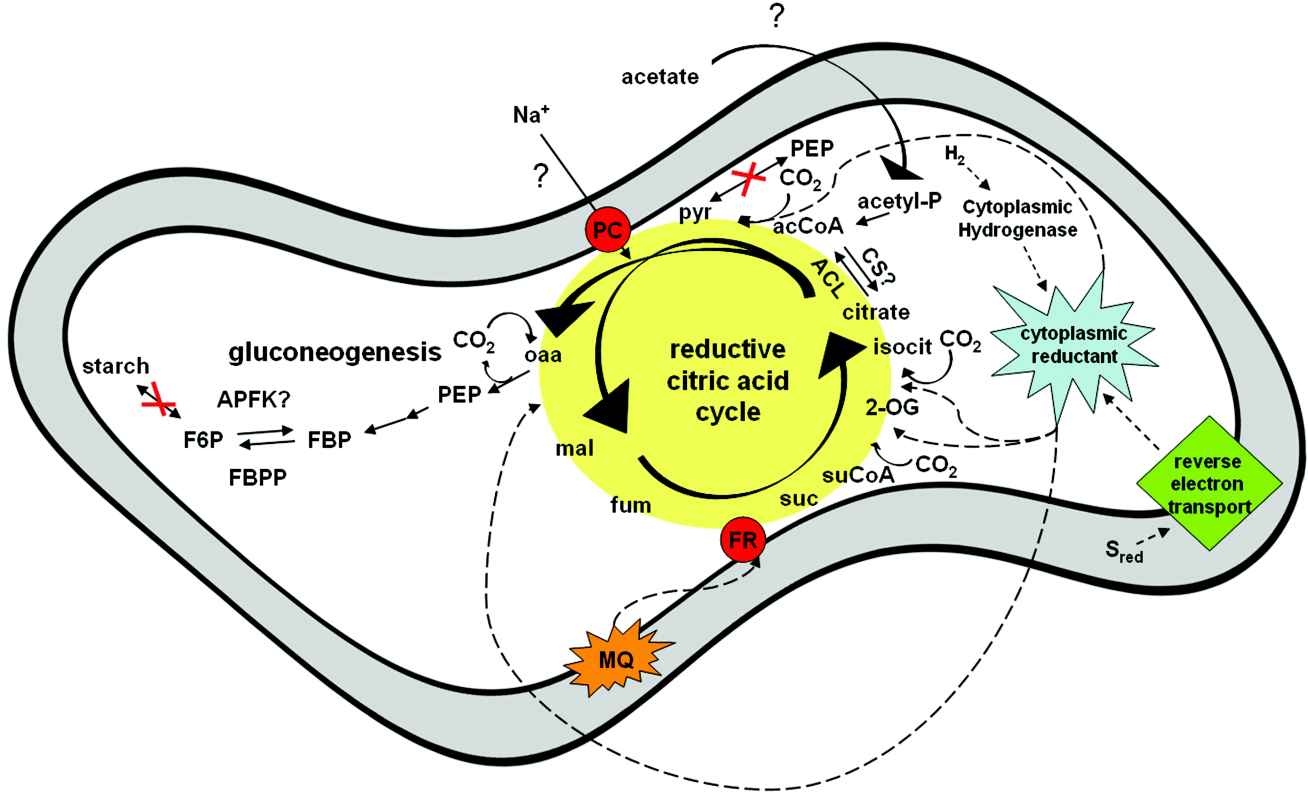
944 *Thiomicrospira crunogena* XCL-2 and *Nitrosococcus oceani* ATCC 19707.

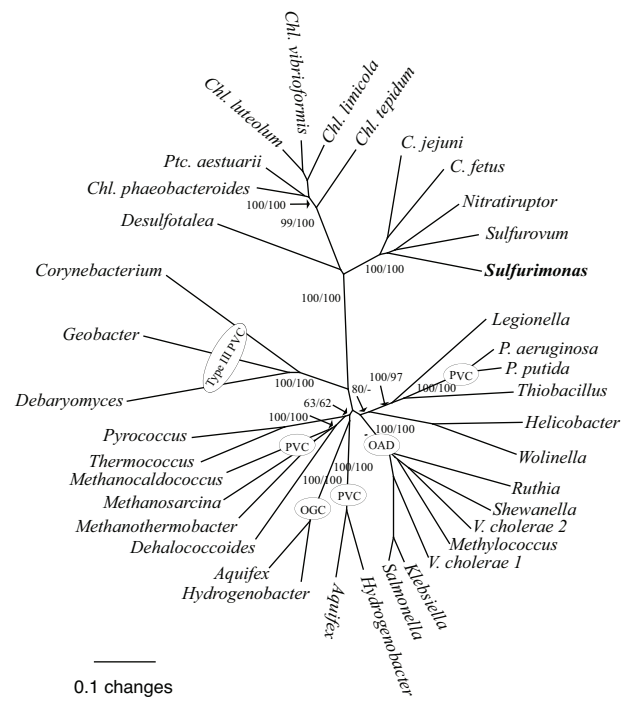
945

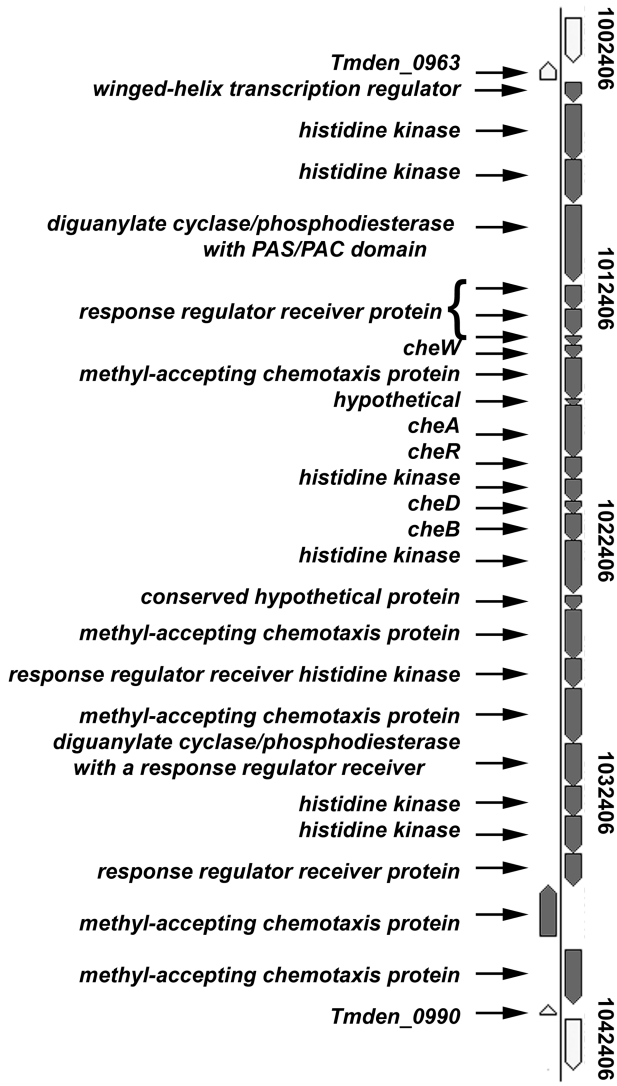
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SUPPLEMENTAL MATERIAL

Further details of genome structure. In addition to the large transposon interrupting one of the flagellar biosynthetic operons (Fig. S1), another transposase gene (*Tmden_1713*) is located near a tRNA^{Thr} gene, adjacent to a hypothetical protein gene (*Tmden_1712*) whose 3'-end is 82% identical, at the nucleotide level, with two transposase genes located downstream (*Tmden_1724* and *Tmden_1725*). These two genes, and regions 5' and 3' of each (totaling 1302 bp apiece), are 100% identical to each other. This region also includes genes encoding a recombinase and phage integrase (*Tmden_1723*; Fig. S2); the presence of the phage integrase gene, identical repeats, and juxtaposition to a tRNA gene suggest that this portion of the genome may be a remnant of a degraded or partially excised prophage. Other potential transposase genes are present (*Tmden_0961*; *Tmden_1698*; *Tmden_1708*), but have insufficient sequence similarity to known proteins for deducing their function convincingly.

Twelve phage integrase genes are present. As expected for phage genes, six of these are near tRNA genes (*Tmden_0248*, *Tmden_0779*, *Tmden_0800*, *Tmden_1618*, *Tmden_1723*; *Tmden_1743*) which are common insertion sites for lysogenic phages (1), and three are flanked by clusters of genes encoding hypothetical proteins (*Tmden_1247*; *Tmden_1618*; *Tmden_1633*), which is consistent with the observation that many phage genes are unique and uncharacterized (2). Two phage integrase genes (*Tmden_0938*; *Tmden_0959*) flank genes encoding a type I restriction modification system gene cluster and are part of a larger region (bp 977850 – 1002764; 5 o'clock, Fig. 1), that have a negative G+C anomaly (31.1%). The remaining two are included in the large transposons described above (*Tmden_0693* and *Tmden_1590*).

24 Another negative G+C anomaly, which also has a GC skew anomaly, is visible on
25 the genome map at approximately 1 o'clock (bps 192095 – 210856; 30.5% G+C; Fig. 1).
26 Although this region contains several genes encoding hypothetical proteins, as one might
27 expect were it derived from a phage, it does not appear to include any transposase or
28 integrase genes, nor does it include any repeated sequences that might suggest recent
29 gene rearrangement in this region.

30 **Restriction-modification systems.** *S. denitrificans* has numerous restriction-
31 modification (RM) systems encoded in its genome. Eleven DNA methyltransferase genes
32 are present, and encode methyltransferases similar to those found in Type I
33 (*Tmden_0697*, *Tmden_0942*, *Tmden_1594*), Type II (*Tmden_0121*; *Tmden_0129*;
34 *Tmden_0130*; *Tmden_0478*; *Tmden_0537*; *Tmden_1565*; *Tmden_1839*; *Tmden_1855*)
35 and Type III (*Tmden_1355*) restriction-modification systems. For 6 of these
36 methyltransferases, genes encoding restriction enzymes are nearby (Type I:
37 *Tmden_0700*; *Tmden_0948*; *Tmden_1597*; Type II: *Tmden_128*; *Tmden_1854*; Type III:
38 *Tmden_1350*) and for two of them the genes appear to encode fused methylase/restriction
39 enzymes (*Tmden_0478*, *Tmden_0537*)(5). Based on genome sequence data, such large
40 numbers of RM systems are not unusual for epsilonproteobacteria: *Helicobacter pylori*
41 has 24 RM systems (3), *C. jejuni* has 10, and *W. succinogenes* has 5 (4, 5). If active,
42 perhaps in *S. denitrificans* these systems provide a robust defense against the introduction
43 of phage and other 'non-native' DNA into the genome.

44

44 **REFERENCES**

45

46 1. **Canchaya, C., G. Fournous, and H. Brussow.** 2004. The impact of prophages
47 on bacterial chromosomes. *Molec Microbiol* **53**:9-18.

48 2. **Hambly, E., and C. A. Suttle.** 2005. The virosphere, diversity, and genetic
49 exchange within phage communities. *Curr Opin Microbiol* **8**:444-450.

50 3. **Kong, H., L. Lin, N. Porter, S. Stickel, D. Byrd, J. Posfai, and R. j. Roberts.**
51 2000. Functional analysis of putative restriction-modification system genes in the
52 *Helicobacter pylori* J99 genome. *Nucl Acids Res* **28**:3216-3223.

53 4. **Roberts, R. J., T. Vincze, J. Posfai, and D. Macelis.** 2007. REBASE--enzymes
54 and genes for DNA restriction and modification. *Nucl Acids Res* **35**:D269-D270

55 5. **Roberts, R. J., T. Vincze, J. Posfai, and D. Macelis.** 2005. REBASE Restriction
56 enzymes and DNA methyltransferases. *Nucl Acids Res* **33**:D230-232.

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58

59 **FIGURE LEGENDS.**

60

61 FIG. S1. Two large identical transposons from the *Sulfurimonas denitrificans*
62 genome. Numbers indicate the position of the regions, in nucleotides, with respect to the
63 origin of replication, and the arrows indicate the presence of the inverted repeat
64 sequences at each end: > = TGTCATTTACAA; < = TTGTAAATGACA.

65

66 FIG. S2. Map of a region from the *Sulfurimonas denitrificans* genome that
67 includes a small repeated region. The duplicate copies of this repeat include the two
68 adjacent transposase genes (shaded grey), while a third region with a high level of
69 identity (82%) is included within a hypothetical gene upstream (also shaded grey).
70 Numbers indicate the position of the regions, in nucleotides, with respect to the origin of
71 replication.

72

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74 FIG. S3. Phylogenetic relationships of NosZ from different bacteria and the
75 archaeon *Pyrobaculum calidifontis*. Suden_1298 is part of a novel nos cluster previously
76 identified in *Wolinella succinogenes* (63). All epsilonproteobacterial sequences have a C-
77 terminal extension and contain a heme *c*-binding motif. The sequences from
78 *Dechloromonas aromatica* and *Magentospirillum magnetotacticum* also have a
79 (somewhat shorter) C-terminal extension, but are lacking a heme *c*-binding motif (63)
80 Sequences were aligned using the program package MacVector. Neighbor-joining and
81 Parsimony trees based on the predicted amino acid sequences were calculated using

82 PAUP 4.0b10. Bootstrap values (1,000 replicates) for the major nodes are given for the
83 neighbor-joining (first value) and parsimony analyses (second value).

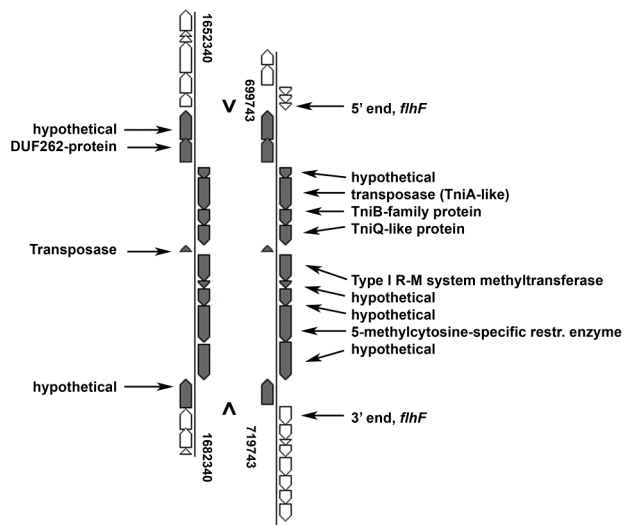
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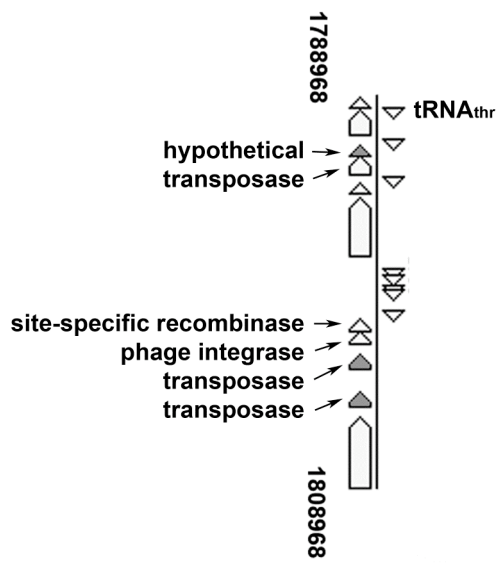
94 FIG. S1

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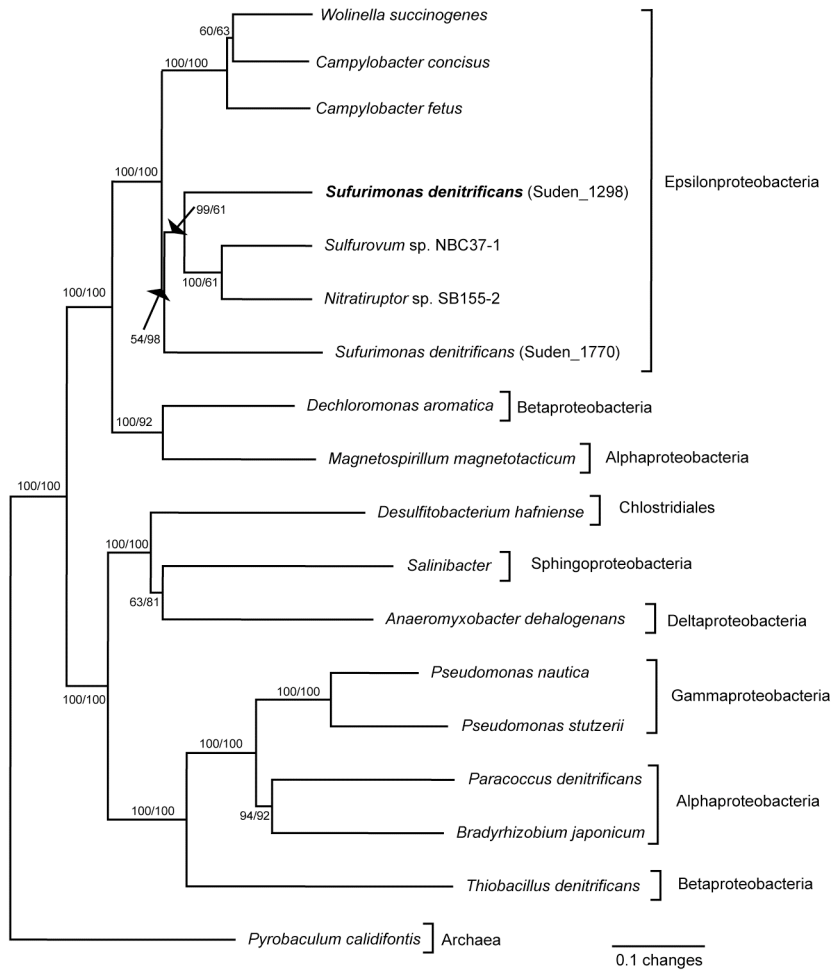
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105 FIG. S2

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113 FIG. S3