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**Desulfamplus magnetovallimortis gen. nov., sp nov., a magnetotactic bacterium from a brackish desert spring able to biomineralize greigite and magnetite, that represents a novel lineage in the Desulfobacteraceae**

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1 ***Desulfamplus magnetovallimortis* gen. nov., sp. nov., a magnetotactic bacterium from**  
2 **a brackish desert spring able to biomineralize greigite and magnetite, that**  
3 **represents a novel lineage in the *Desulfobacteraceae***

4

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22 **ABSTRACT**

23 A magnetotactic bacterium, designated strain BW-1<sup>T</sup>, was isolated from a brackish spring  
24 in Death Valley National Park (California, USA) and cultivated in axenic culture. The  
25 Gram-negative cells of strain BW-1<sup>T</sup> are relatively large and rod-shaped and possess a  
26 single polar flagellum (monotrichous). This strain is the first magnetotactic bacterium  
27 isolated in axenic culture capable of producing greigite and/or magnetite nanocrystals  
28 aligned in one or more chains per cell. Strain BW-1<sup>T</sup> is an obligate anaerobe that grows  
29 chemoorganoheterotrophically while reducing sulfate as a terminal electron acceptor.  
30 Optimal growth occurred at pH 7.0 and 28°C with fumarate as electron donor and carbon  
31 source. Based on its genome sequence, the G + C content is 40.72 mol %. Phylogenomic  
32 and phylogenetic analyses indicate that strain BW-1<sup>T</sup> belongs to the *Desulfobacteraceae*  
33 family within the *Deltaproteobacteria* class. Based on average amino acid identity, strain  
34 BW-1<sup>T</sup> can be considered as a novel species of a new genus, for which the name  
35 *Desulfamplus magnetovallimortis* is proposed. The type strain of *Desulfamplus*  
36 *magnetovallimortis* is BW-1<sup>T</sup> (JCM 18010<sup>T</sup> - DSM 103535<sup>T</sup>).

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41 Key words: *Desulfamplus magnetovallimortis*, BW-1, magnetotactic bacteria, greigite,  
42 magnetite, magnetotaxis, magnetosome, *Deltaproteobacteria*, sulfate-reducing bacteria.

43

## 44 **Introduction**

45 Magnetotactic bacteria represent a phylogenetically, ecologically and  
46 metabolically diverse group of prokaryotes able to biomineralize single-magnetic-domain  
47 nanocrystals of magnetite (an iron oxide (Fe(II)Fe(III)<sub>2</sub>O<sub>4</sub>)) or greigite (an iron sulfide  
48 (Fe(II)Fe(III)<sub>2</sub>S<sub>4</sub>)) in specific organelles [5]. These organelles, called magnetosomes, are  
49 aligned in one or more chains within the cytoplasm and cause cells to actively swim  
50 along the Earth's magnetic field lines [19]. This behavior, termed magnetotaxis, makes  
51 the search for optimal concentrations of certain nutrients and redox conditions within  
52 water columns and sediments more efficient by simplifying a three-dimensional search to  
53 a linear search [19].

54 Although magnetotactic bacteria were discovered 40 years ago [11], relatively  
55 few strains have been cultivated in pure culture. Officially described and cultured  
56 magnetotactic species are all magnetite-producers and belong to the genera  
57 *Magnetospirillum* [36,37,41], *Desulfovibrio* [40], *Magnetovibrio* [10], *Magnetococcus*  
58 [9] and *Magnetospira* [55]. The candidate genus name of *Magnetoglobus* has also been  
59 officially proposed for a magnetotactic multicellular prokaryote [2].

60 Greigite-producing magnetotactic bacteria were first described in 1983 [17] but  
61 the mineral composition of the crystals in the magnetosomes of these organisms was only  
62 identified in 1990 [16,35]. Until recently, they were thought to be confined to marine  
63 habitats [45]. There are two known morphological types of greigite-producers: a group of  
64 morphologically unique, multicellular magnetotactic prokaryotes (MMPs), and a group of  
65 large rod-shaped bacteria [46]. While a good deal of information has been acquired  
66 regarding the MMPs from culture-independent, environmental [4,8,44,53,57] and  
67 genomics studies [1,3,24], their isolation in axenic culture has not yet been successful,  
68 although *Candidatus Magnetoglobus multicellularis* was co-cultured in a medium where  
69 cells reduced sulfate and used succinate as an electron donor [3]. The MMP group  
70 represents an assemblage of obligately multicellular organisms that consist of about 10-  
71 60 genetically identical cells and is phylogenetically affiliated with the  
72 *Desulfobacteraceae* family within the *Deltaproteobacteria* class of the *Proteobacteria*

73 phylum [13,14,44,53,57,58]. The large rod-shaped greigite and/or magnetite-producing  
74 bacteria have been studied for several decades using culture-independent techniques from  
75 environmental samples [6,7,21,43,45] but have only been phylogenetically identified  
76 [30,52] and cultured [30] recently. The greigite-producing, rod-shaped bacteria also  
77 belong to the *Desulfobacteraceae* family [30].

78         The first isolation and cultivation of a large, rod-shaped greigite-producing  
79 magnetotactic bacterium, referred to as *Candidatus Desulfamplus magnetomortis*  
80 (renamed here *Desulfamplus magnetovallimortis*) strain BW-1, was recently described  
81 [30]. *In silico* analyses of this organism revealed the presence of two different  
82 magnetosome gene clusters. Based on comparative genomics, one magnetosome gene  
83 cluster was proposed to be responsible for greigite biomineralization and the other for  
84 magnetite synthesis [30,32]. The presence of two similar clusters was also identified in  
85 the MMP *Ca. Magnetomorum* strain HK-1 collected from samples from the North Sea,  
86 Germany, supporting the ability of this bacterium to synthesize magnetite and greigite  
87 magnetosomes. Magnetosome genes involved in greigite formation were first described  
88 in *Ca. Magnetoglobus multicellularis* [1]. Finally, greigite magnetosome genes were also  
89 identified in a draft genome of an uncultivated bacterium belonging to the candidate  
90 phylum *Latescibacteria* [34]. Although there is no evidence that this bacterium is able to  
91 produce greigite, it is possible that greigite biomineralization extends to this phylum as  
92 well as the *Deltaproteobacteria*.

93         *Desulfamplus magnetovallimortis* strain BW-1<sup>T</sup> (Bad Water-1) is the subject of  
94 the current study. This magnetotactic bacterium was isolated from a brackish spring at  
95 Badwater Basin, Death Valley National Park, California, USA [30]. Using cultivation  
96 experiments and genomic approaches, here we fully characterize and name this strain that  
97 represents the first greigite-producing magnetotactic bacterium isolated in axenic culture.

98

## 99 **Materials and methods**

100 *Sample collection, enrichment and purification*

101 Strain BW-1<sup>T</sup> was isolated from mud and water collected from the brackish spring  
102 located at Badwater Basin in Death Valley, California, USA [30]. For isolation of strain  
103 BW-1, cells of magnetotactic bacteria were first magnetically concentrated from water  
104 samples using the magnetic capillary racetrack technique [56] and then inoculated into a  
105 modified semisolid, oxygen concentration gradient ([O<sub>2</sub>]-gradient) medium based on a  
106 growth medium originally designed for *Desulfobacterium vacuolatum*. The medium  
107 consisted of an artificial seawater (ASW) base diluted to the approximate salinity of the  
108 water sample, containing (per liter): NaCl, 20 g; MgCl<sub>2</sub>•6H<sub>2</sub>O, 3 g; Na<sub>2</sub>SO<sub>4</sub>, 3 g; KCl, 0.5  
109 g; and CaCl<sub>2</sub>•2H<sub>2</sub>O, 0.2 g. To this was added (per liter) the following, in order, prior to  
110 autoclaving: 50 μL of 0.2% (wt/vol) aqueous resazurin, 5 mL of modified Wolfe's  
111 mineral elixir [19], 0.3 g of NH<sub>4</sub>Cl, 2.4 g of HEPES, 2 g of Casamino acids and 2 g of  
112 Agar Noble (both from Difco). The pH of the medium was then adjusted to 7.0 prior to  
113 autoclaving. Once the medium cooled to about 45°C, the following solutions were added  
114 (per liter), in order, from stock solutions (except for the cysteine, which was made fresh  
115 and filter sterilized directly into the medium): 0.5 mL of vitamin solution [19]; 1.8 mL of  
116 0.5 M KHPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub> buffer, pH 7.0; 3 mL of 0.01 M FeCl<sub>2</sub> dissolved in 0.02 N HCl;  
117 and L-cysteine to give a final concentration of 0.4 g/L. The medium (10 mL) was  
118 dispensed into sterile, 15 x 125 mm screw-capped test tubes. All cultures were incubated  
119 at 28°C and, after approximately one week, an aggregate of cells formed in the anaerobic  
120 zone of the medium. The large rod-shaped cells were not magnetotactic in this medium  
121 and it was only when they were transferred in a specific liquid medium that cells became  
122 magnetotactic. An axenic culture of strain BW-1<sup>T</sup> was obtained after three successive  
123 rounds of dilution to extinction in this latter medium. Purity of the cultures was  
124 determined using light microscopy and by amplification and sequencing of the 16S rRNA  
125 gene.

#### 126 *Media and culture conditions*

127 For magnetosome formation, cells of strain BW-1<sup>T</sup> were grown in BWM medium  
128 in 250 mL Schott bottles containing 100 mL of liquid anaerobic medium closed by a cap  
129 and a rubber stopper. The formula of the medium was (per liter): 20 g NaCl, 3 g  
130 MgCl<sub>2</sub>•6H<sub>2</sub>O, 3 g Na<sub>2</sub>SO<sub>4</sub>, 0.2 g CaCl<sub>2</sub>•2H<sub>2</sub>O and 0.5 g KCl. To a liter of the basal

131 medium, 5 ml modified Wolfe's mineral elixir [19], 50  $\mu$ L 1% aqueous resazurin, 0.3 g  
132  $\text{NH}_4\text{Cl}$ , 2.4 g 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 1 g  
133 fumaric acid were added and the pH adjusted to 7.5 with 10 M NaOH. The medium was  
134 then autoclaved. After autoclaving, 0.5 mL of an anaerobic stock solution of vitamins  
135 [19]; 1.8 mL of 0.5 M  $\text{KHPO}_4$  buffer (pH 7.0); 2 mL or 10 mL of 10 mM  $\text{FeCL}_2 \cdot 4\text{H}_2\text{O}$   
136 (in 0.02 M HCl) and 0.4 g of freshly made filtered sterilized cysteine were added to the  
137 medium. The medium was then bubbled with  $\text{O}_2$ -free  $\text{N}_2$  for 30 min. The medium was  
138 inoculated with 5 mL of a culture in exponential growth phase and incubated at 22°C.

139         Electron donors and acceptors were tested in this growth medium. The following  
140 compounds were tested as terminal electron acceptors (sodium salts, when appropriate):  
141 nitrate (2 mM), nitrite (2 mM), fumarate (20 mM), trimethylamine oxide (TMAO; 15  
142 mM), dimethylsulfoxide (DMSO; 15 mM), sulfur (5 mM), sulfite (5 mM), thiosulfate (5  
143 mM), tetrionate (5 mM), dithionite (5 mM) and  $\text{N}_2\text{O}$  (1 atm in the headspace). Except  
144 for the tubes containing nitrous oxide ( $\text{N}_2\text{O}$ ), all others were flushed with  $\text{O}_2$ -free  $\text{N}_2$ . To  
145 test for heterotrophic growth, potential carbon sources were added to tubes to give a final  
146 concentration of 0.1% (wt/vol or vol/vol) using the BWM liquid medium minus fumaric  
147 acid. The following carbon sources were tested (sodium salts for acids, L-enantiomers  
148 were used for amino acids, D-enantiomers for sugars): alanine, aspartic acid, glutamic  
149 acid, glutamine, glycine, isoleucine, leucine, proline, serine, valine, butanol, glucose,  
150 casamino acids, yeast extract, butyrate, malate, pyruvate, acetate, fumarate, gluconic acid,  
151 lactic acid and succinate. To test for autotrophic growth, fumaric acid was omitted from  
152 the medium, 2.8 mL of a solution of 0.8 M  $\text{NaHCO}_3$  (autoclaved dry; sterile water added  
153 after autoclaving to make the fresh stock solution) was added to the medium which was  
154 then bubbled with  $\text{H}_2$  for 30 min. If growth was observed, the culture was transferred  
155 twice further in the same medium in triplicate. Fumaric acid was used as the positive  
156 control and sterile  $\text{H}_2\text{O}$  was used as the negative control. Tubes were incubated for at  
157 least 3 weeks at 28°C.

158 *Whole genome sequencing and annotation*

159 Whole genome sequencing of *Desulfamplus magnetovallimortis* strain BW-1 was  
160 previously described [31]. The draft genome sequence partially annotated was submitted  
161 to the European Nucleotide Archive and carries the accession number PRJEB14757. The  
162 genome was assembled using the MicroScope platform resulting in 108 scaffolds and 346  
163 contigs for a total length of 6,784,998 bases. Automatic and manual sequence annotations  
164 were performed using the MicroScope platform from the Genoscope [51]. There are  
165 11.28% of Nosferatu Repeated Regions (excluding undetermined bases). CDS length is  
166 about 942.13 bp with an intergenic length of 210 bp. A total of 6,137 CDS are present in  
167 the genome.

#### 168 *Phylogenetic analysis*

169 Evolutionary relationships between *Desulfamplus magnetovallimortis* BW-1<sup>T</sup> and  
170 its relatives were investigated at both 16S rRNA sequence and whole genome resolutions.  
171 First, genomes of several *Deltaproteobacteria* type strains were downloaded from the  
172 public database NCBI (updated up to March 2016) and uploaded into the MicroScope  
173 platform. These included at least one representative of each order (*Desulfobacterales*,  
174 *Desulfarculales*, *Syntrophobacterales*, *Myxococcales*, *Bdellovibrionales*,  
175 *Desulfuromonadales*, *Desulfurellales* and *Desulfovibrionales*) with the exception of the  
176 *Desulfobacterales* and *Desulfovibrionales*, for which several genomes were included in  
177 the dataset. A core genome (*i.e.*, genes detected in all genomes compared, for which  
178 protein sequences shared > 50% identity over > 80% of their length), was defined with  
179 the Microscope comparative genomics tool and categorized into several MicroScope gene  
180 families (MICFAM). Among the 131 MICFAM shared by the 24 genomes, 70 were  
181 represented by a unique sequence per genome and 32 out of those coded for ribosomal  
182 proteins. Core amino acids sequences were aligned independently using MAFFT 7 [23].  
183 Alignments were trimmed to remove positions composed of more than 95% gaps and  
184 concatenated into a single alignment of 23,813 amino acids positions among which  
185 18,161 were polymorphic. A maximum-likelihood (ML) tree was built with RAxML  
186 8.2.6 [47] under the GAMMA model of rate heterogeneity using empirical AA  
187 frequencies and the LG substitution model (selected with the Bayesian criterion). A total  
188 of 200 bootstrap replicates automatically determined by the MRE-based bootstopping



189 criterion were conducted under the rapid bootstrapping algorithm, among which 100 were  
190 sampled to generate proportional support values.

191 Only few genomes of members of the *Desulfobacteraceae* family are currently  
192 available. Thus, a phylogenetic tree based on the 16S rRNA gene sequences was built to  
193 determine the relatedness of *Desulfamplus magnetovallimortis* BW-1<sup>T</sup> within the existing  
194 diversity of *Desulfobacteraceae* species already described. For this purpose, 43  
195 sequences of cultured type strains > 1,200 bp available in the Ribosomal Database Project  
196 (<https://rdp.cme.msu.edu>) were downloaded, aligned and trimmed as described above. A  
197 ML tree was also built following the same procedure than described above but under a  
198 GAMMA model of rate heterogeneity, estimating a Generalized Time Reversible (GTR)  
199 model for nucleotide substitution and an estimate of the proportion of invariable sites.

#### 200 *Chemotaxonomic analyzes*

201 For fatty acid and respiratory quinones analyses, sulfate-reducing cells of strain  
202 BW-1<sup>T</sup>, grown in liquid medium with fumarate as electron donor were harvested during  
203 late exponential phase. Fatty acid analysis and respiratory quinones was carried out by  
204 the Identification Service of the Deutsche Sammlung von Mikroorganismen und  
205 Zellkulturen GmbH (DSMZ), Braunschweig, Germany. Fatty acids were extracted,  
206 purified, methylated and quantified by gas chromatography using the standard Microbial  
207 Identification System [22,27,38]. Respiratory quinones were analyzed by thin layer  
208 chromatography using the standard Microbial Identification System [49,50].

#### 209 *Electron microscopy*

210 Transmission electron microscopy (TEM) of whole cells was done using a Tecnai (FEI  
211 Company, Hillsboro OR) model G2 Biotwin electron microscope using an accelerated  
212 voltage of 100 kV. High resolution transmission microscopy (HRTEM) was carried out  
213 on a Jeol 2100F microscope. This machine, operating at 200 kV, is equipped with a  
214 Schottky emission gun, an ultra-high-resolution pole piece, and an ultrathin window  
215 JEOL X-ray Energy Dispersive Spectrometer. HRTEM images were obtained with a  
216 Gatan US 4000 CCD camera.

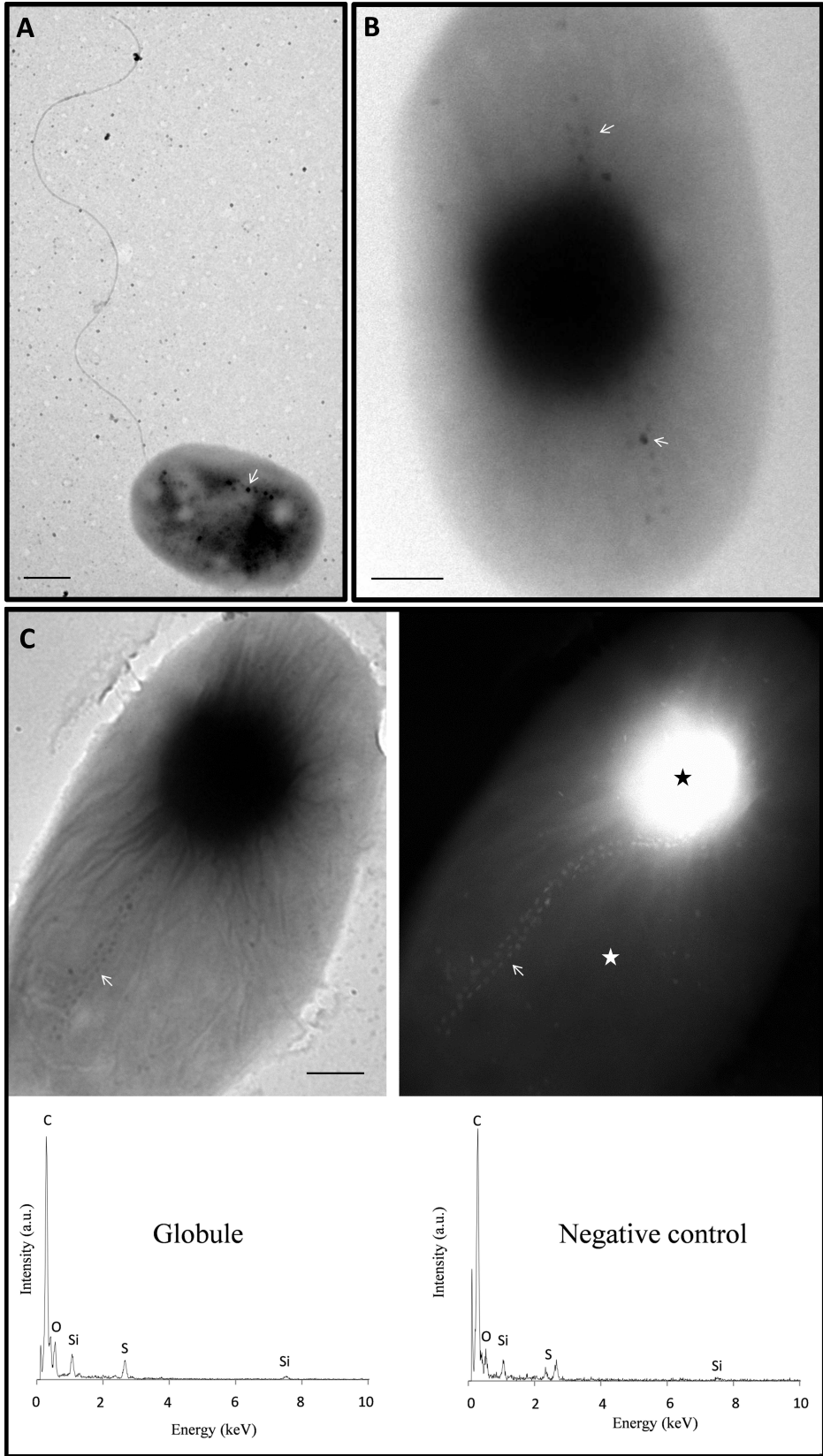
217 A drop of culture containing cells of strain BW-1<sup>T</sup> was deposited onto TEM  
218 copper grids coated with a carbon film, which were then washed and dried in air. The  
219 grids were stained with 1% uranyl acetate in particular to observe flagella.

220

## 221 **Results and discussion**

### 222 *Cell morphology*

223 Light microscopy and TEM observations show that cells of strain BW-1<sup>T</sup> are  
224 relatively large, rod-shaped and possess a single polar flagellum that measures 3-4 times  
225 the cell's body length (Fig. 1A). Although the majority of the cells are not motile in the  
226 lag phase, most of the cells in the exponential and stationary phases are motile. Cells  
227 have a smooth motility in the presence or absence of a magnetic field with an  
228 approximate speed of 30  $\mu\text{m}\cdot\text{s}^{-1}$ . Cultured cells synthesize a large electron-dense  
229 inclusion of unknown composition approximately 500 nm in diameter (Fig. 1B) that were  
230 not observed in cells collected from the environment. Specific chemical elements in this  
231 inclusion were not detected using energy-dispersive X-ray spectroscopy (Fig. 1C). This  
232 inclusion was more commonly observed in cells in stationary or early exponential growth  
233 phases.



235 **Fig. 1.** Transmission electron microscope (TEM) images of whole cells of strain BW-1<sup>T</sup> showing (A) the  
 236 single polar flagellum and (B) the large inclusion of unknown composition and function. (C) TEM and  
 237 scanning-transmission electron microscope (STEM) images showing a large inclusion analyzed with  
 238 energy-dispersive X-ray spectroscopy. Elemental spectrum of the inclusion (left; black star inside the  
 239 inclusion) compared to that of background (right; white star outside the inclusion) showing that the  
 240 inclusion is not composed by specific elements. White arrows indicate magnetosomes. Scale bars represent  
 241 200 nm.

242

### 243 *Growth characteristics*

244 Cells growing in BWM medium appeared to be more motile and contained more  
 245 magnetosomes than other growth media tested (see Magnetosomes section). To increase  
 246 the growth rate, yeast extract was added (0.2 g/L) to BWM medium and the cultures  
 247 incubated at 28°C. Under such conditions, cells were still motile but did not show any  
 248 magnetic response under the light microscope and only biomineralized weakly  
 249 crystallized particles as observed by TEM. The minimum doubling time during  
 250 exponential growth phase in modified BWM medium was about 6-8 hours. Physiological  
 251 as well as morphological properties of strain BW-1<sup>T</sup> are listed in Table 1 and compared to  
 252 closely related species.

253

254 **Table 1.** Selected characteristics that differentiate strain BW-1<sup>T</sup> from its closest relatives.

255 NR, Not reported; +, good growth; (+), poor growth; -, no growth. Reference strains: 1, *Desulfobacterium*  
 256 *autotrophicum* (data from [48] and [12]); 2, *Desulfobacula toluolica* (data from [26]); 3, *Desulfotignum*  
 257 *balticum* (data from [26]); 4, *Desulfospira joergensenii* (data from [18]); 5, *Desulfoconvexum algidum*  
 258 (data from [25]); *Desulfobacter postgatei* (data from [54]).

Characteristic	BW-1 <sup>T</sup>	1	2	3	4	5	6
Cell morphology	Rod to oval	Oval	Oval	Rod	Vibrioid	Curved/vibrioid	Rod to ellipsoid
Magnetotactic	Yes	No	No	No	No	No	No
G-C content (mol%)	41	49	41	62	50	46	46

Optimum salinity (g NaCl L <sup>-1</sup> )	15-25		20	20	12-20	20-30	5
Optimum temperature (°C)	28	25-28	28	28-32	26-30	14-16	32
Compounds used as electron donor and carbon source:							
H <sub>2</sub> + CO <sub>2</sub>	-	+	-	(+)	+	+	-
Formate	-	+	-	(+)	+	+	-
Acetate	-	+	-	(+)	-	-	+
Lactate	+	+	-	+	+	+	-
Pyruvate	+	+	+	+	+	-	-
Fumarate	+	+	+	+	+	+	-
Succinate	+	+	+	(+)	+	-	-
Malate	+	+	+	+	-	+	-
Fermentative growth	-	+	NR	+	-	+	-
Electron acceptors:							
Sulfite	-	-	NR	+	+	-	+
Sulfur	-	-	NR	NR	+	+	-
Thiosulfate	+	+	NR	+	+	+	+
Nitrate	-	-	NR	-	-	-	-

259

260 Cells of strain BW-1<sup>T</sup> grew anaerobically in the presence of sulfate and  
 261 thiosulfate, but not with nitrate, nitrite, fumarate, TMAO, DMSO, sulfite, sulfur,  
 262 tetrathionate, dithionite and N<sub>2</sub>O as terminal electron acceptors.

263 Because some magnetotactic strains of *Desulfovibrio* were recently shown to  
 264 respire with O<sub>2</sub> [29], we tested BW-1<sup>T</sup> for this ability in an [O<sub>2</sub>]-gradient medium where  
 265 fumaric acid was added as the electron donor. In presence of sulfate, cells of strain BW-  
 266 1<sup>T</sup> only grew as aggregates below the oxic-anoxic interface. In absence of sulfate, no  
 267 growth was observed showing their inability to use O<sub>2</sub> as electron acceptor. Strain BW-1<sup>T</sup>  
 268 represents, to date, the only cultured magnetotactic bacterium unable to grow  
 269 microaerobically raising questions on the role(s) of magnetosomes in this bacterium. It is  
 270 possible that the conditions for aerobic growth of BW-1<sup>T</sup> remain to be elucidated or that  
 271 this ability was lost due to long term transfer of the culture under strictly anaerobic  
 272 conditions. Moreover, as it was reported for *Desulfobacterium autotrophicum* [48], strain  
 273 BW-1<sup>T</sup> also has in its genome *cydAB* genes coding for a cytochrome d ubiquinol oxidase

274 that could enable strain BW-1<sup>T</sup> to respire oxygen at low concentrations [20,33].  
275 Additionally, strain BW-1<sup>T</sup> possesses a catalase-peroxidase (KatG), a selenocysteine-  
276 containing peroxidase, rubrerythrin (Rbr) and a rubredoxin oxidoreductases (Rbo) for  
277 oxygen detoxification.

278 Glutamate, glutamine, casamino acids, yeast extract, fumarate, lactate, malate,  
279 pyruvate and succinate supported growth and are thus potential carbon sources for BW-  
280 1<sup>T</sup> (Table 1).

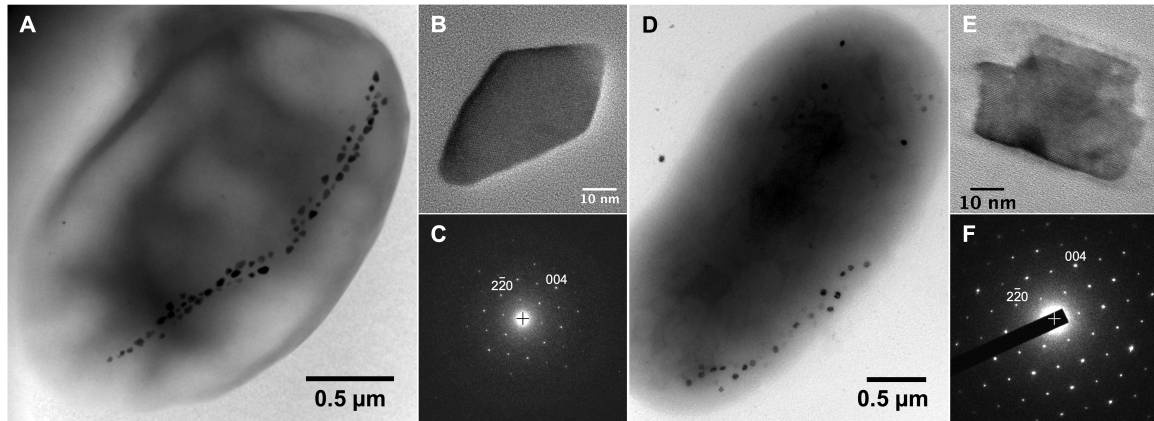
281 In BWM medium, cultures in exponential to late-exponential growth phase can be  
282 stored for several weeks at 4°C before having to be transferred into fresh medium. For  
283 long term storage, concentrated cells remained viable for several years in BWM medium  
284 supplemented with 10% (final concentration) dimethylsulfoxide (DMSO) at -80°C.

#### 285 *Magnetosome biomineralization*

286 Individual cells of strain BW-1<sup>T</sup> grown in BWM medium contained one or two  
287 magnetosome chains composed of magnetite and/or greigite (Fig. 2A and 2B). Magnetite  
288 crystals produced by BW-1<sup>T</sup> were consistently bullet-shaped, whereas greigite crystals  
289 were pleomorphic. When a cell biomineralizes magnetite and greigite magnetosomes,  
290 both types of particles appear to be in the same chain likely sharing the same MamK  
291 filament. We previously showed that the composition of magnetosome mineral crystals is  
292 correlated with the concentration of hydrogen sulfide produced during growth (sulfate-  
293 reduction) and affected by external environmental conditions, for example, redox  
294 potential [6,30]. Indeed, when sulfide accumulates (> 0.3 mM) in the growth medium,  
295 most magnetosomes contain greigite [30]. Since the initial description of strain BW-1<sup>T</sup>  
296 [26], we have improved *in vitro* magnetosome formation using BWM medium.

297 A relatively high concentration of iron in the medium (> 100 µM) appeared to be  
298 favorable for magnetite rather than greigite formation. It seems that magnetite production  
299 requires a high concentration of iron and a relatively high redox potential/low sulfide  
300 concentration. Under such conditions, hydrogen sulfide, produced during growth and  
301 sulfate reduction, is trapped as FeS by the relatively high concentration of iron in the

302 growth medium. Conversely, greigite formation seems favored at lower iron  
303 concentrations ( $< 20 \mu\text{M}$ ) and low redox potential/high sulfide concentration. Under  
304 these conditions, most of the hydrogen sulfide produced during cell growth is not trapped  
305 and reduces the redox potential of the growth medium.



306

307 **Fig. 2.** Transmission electron microscope (TEM) images of cells of strain BW-1<sup>T</sup>. (A) Cell grown in BWM  
308 medium containing 100 μM iron producing only magnetite magnetosomes, (B) high-resolution TEM  
309 (HRTEM) image of a magnetite crystal and corresponding selected area diffraction (SAED) of crystal  
310 viewed along the [110] zone axis (C). (D) Cell grown in BWM medium containing 20 μM iron, cell  
311 produces only greigite magnetosomes, (E) HRTEM image of greigite crystal and corresponding SAED of  
312 crystal viewed along the [110] zone axis (F).

313

314 Genes involved in magnetosome formation were previously described in detail  
315 [32] and will not be further discussed here. We have grown strain BW-1<sup>T</sup> consecutively  
316 since 2010 under these anaerobic media and cells were always able to produce  
317 magnetosomes. Non-magnetotactic mutant were never obtained in our growth conditions  
318 as it was reported for other magnetotactic species. Indeed, the loss of magnetosome  
319 formation and, thus, magnetotaxis by some cultivated MTB occurs relatively easily in  
320 culture following the loss of magnetosome genes [15,42]. The absence of integrase and/or  
321 transposase genes in close proximity of the magnetosome gene clusters [32] indicates that  
322 this region is relatively stable in the genome of BW-1<sup>T</sup> and could explain why the  
323 magnetotactic phenotype was never lost for this strain.

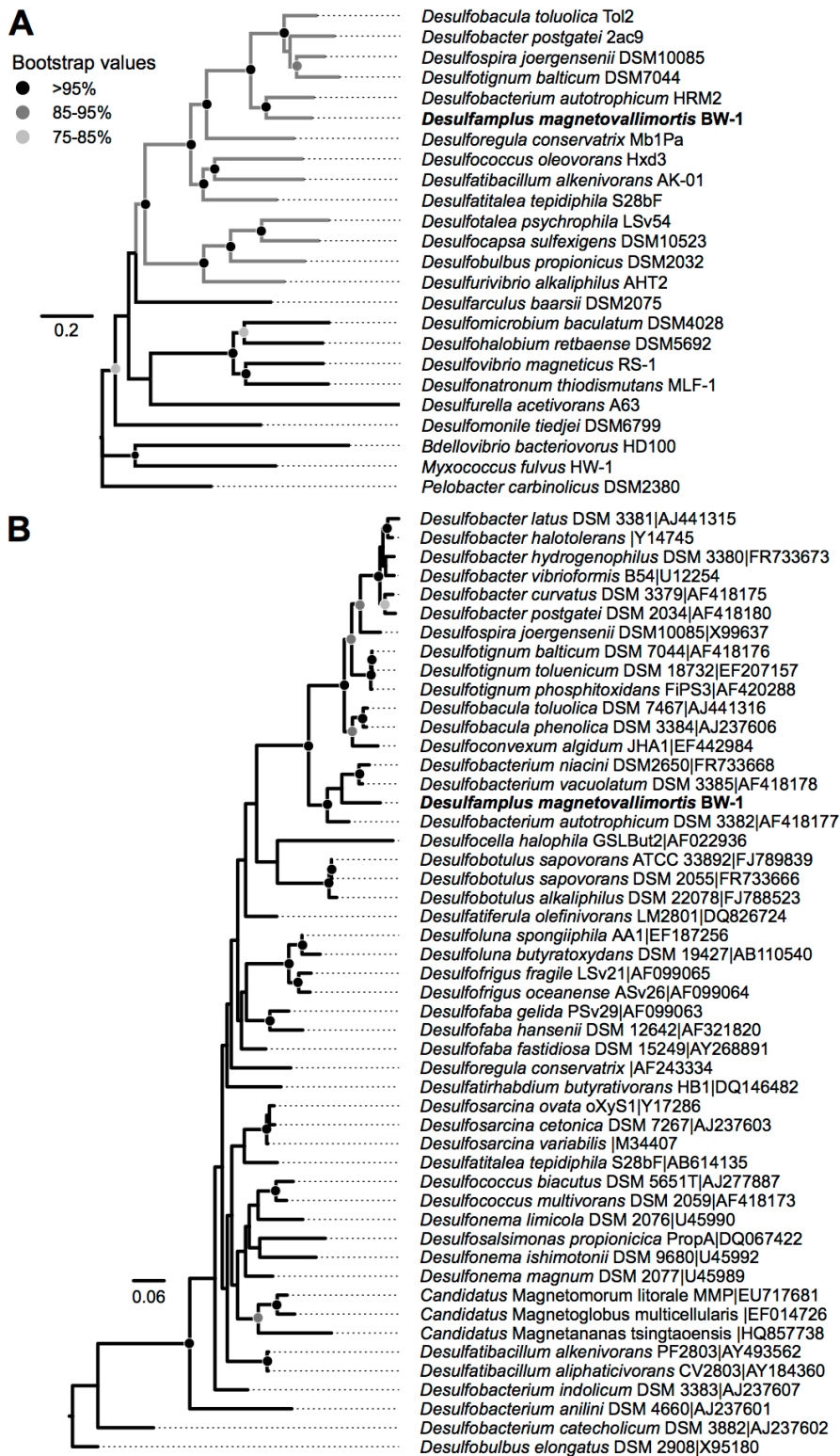
324 *Phylogeny of strain BW-1<sup>T</sup>*

325 Phylogenomics analyses confirmed the placement of strain BW-1<sup>T</sup> in the order  
326 *Desulfobacterales*, more specifically in the family *Desulfobacteraceae* (Fig. 3A). The  
327 three closest 16S rRNA gene sequences from type strains are those of *Desulfobacterium*  
328 *vacuolatum* (93.3%), *Desulfobacterium autotrophicum* (92.0%) and *Desulfobacula*  
329 *toluolica* (89.4). The closest genetically related species to BW-1<sup>T</sup>, for which a whole  
330 genome was sequenced and available, is *Desulfobacterium autotrophicum* HRM2  
331 (=DSM 3382<sup>T</sup>). Amino acid sequences from coding DNA sequences between the two  
332 species are 58.15% identical based on the KostasLab two-way average amino acid  
333 identity (AAI) calculator (<http://enve-omics.ce.gatech.edu/aai/>) (Fig. 4) [39]. Such a  
334 genetic divergence between two genomes is generally found among bacterial strains  
335 belonging to different genera [39]. A 16S rRNA based tree containing BW-1<sup>T</sup> and  
336 relatives confirmed that the most recent ancestor of BW-1<sup>T</sup> is shared with  
337 *Desulfobacterium* species (Fig. 3B). Together with this phylogenetic evidence, ecological  
338 and phenotypic features of BW-1<sup>T</sup> compared to those of its most closely related species  
339 support our contention that BW-1<sup>T</sup> is the first representative of a new genus we name  
340 here as *Desulfamplus*. In addition, among other compared characteristics, no  
341 *Desulfobacterium* spp. were shown to be magnetotactic and magnetosome genes are not  
342 present in the genome of *Desulfobacterium autotrophicum* HRM2, the only genome  
343 sequenced and available from members of this genus [48]. Moreover, only 26-31% of  
344 their genome sequences are shared (Fig. 4).

345 The genus *Desulfamplus* likely contains several species since other magnetotactic  
346 bacteria closely related to BW-1<sup>T</sup> have been described from natural environments  
347 [30,45,52]. This clade appears to consist of at least two groups that could potentially be  
348 divided into two separate genera based on 16S rRNA gene sequence divergence [28,30].  
349 New cultures and new genomes sequenced from those magnetotactic relatives to BW-1<sup>T</sup>  
350 are clearly necessary to determine the number of different genera these groups represent  
351 and how widespread this group of sulfate-reducing bacteria is.

352

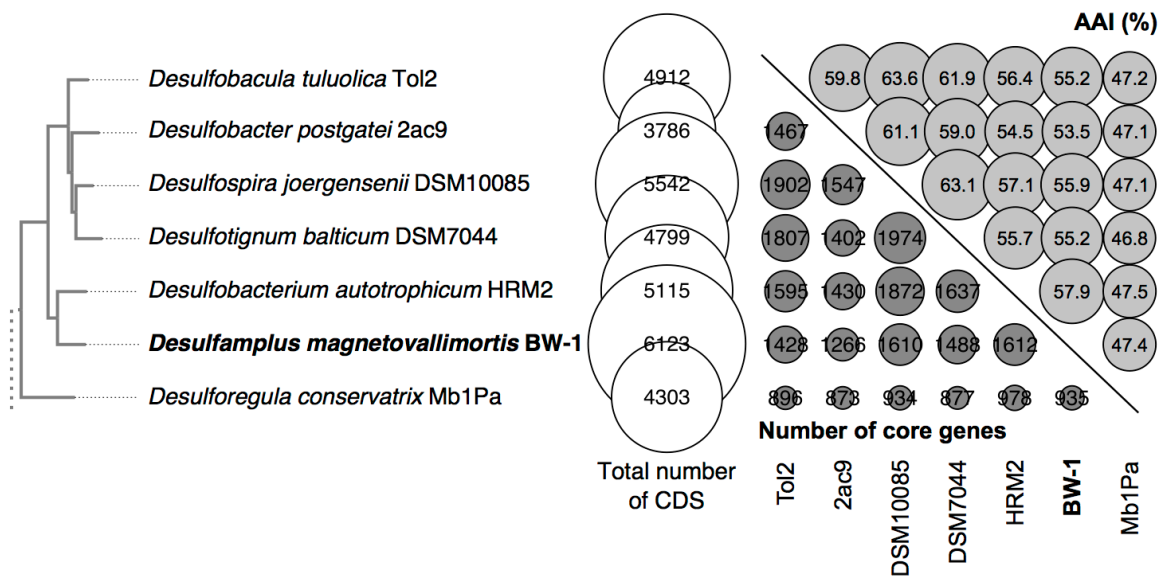




353

354 **Fig. 3.** Phylogenetic position of *Desulfamplus magnetovallimortis* strain BW-1<sup>T</sup> within the  
 355 *Deltaproteobacteria* class. (A) Unrooted ML phylogeny showing the evolutionary relationships between  
 356 *Desulfamplus magnetovallimortis* strain BW-1<sup>T</sup> with 23 sequenced genomes of *Deltaproteobacteria* type

357 strains. The tree was built from the concatenated sequences of 70 aligned core proteins representing a total  
 358 of 26,802 amino acids positions. The tree was inferred with the GAMMA model of rate heterogeneity and  
 359 the LG matrix for describing protein evolution. Grey branches points out specifically the *Desulfobacterales*  
 360 order. (B) ML phylogeny rooted with *Desulfobulbus elongatus* DSM2908 showing the evolutionary  
 361 relationships of *Desulfamplus magnetovallimortis* strain BW-1<sup>T</sup> with 44 *Desulfobacteraceae* type strains  
 362 specifically, based on 1470 aligned bp of 16S rRNA gene sequences. The tree was inferred estimating a  
 363 GTRGAMMA model to describe nucleotide evolution. Trees were drawn to scale and branches length  
 364 represents the number of base substitutions per site. A total of 200 and 500 bootstrap replicates  
 365 automatically determined by the MRE-based bootstopping criterion were conducted for both trees  
 366 respectively under the rapid bootstrapping algorithm to test robustness of the nodes, among which 100 were  
 367 sampled to generate proportional support values.



368

369 **Fig. 4.** Core genome sizes and percentages average amino acid identity (AAI) between *Desulfamplus*  
 370 *magnetovallimortis* BW-1<sup>T</sup> and its closest relatives. Core genes are defined by MaGe as genes detected in  
 371 both genomes, for which coding protein sequences shared a minimum of 50% identity over a minimum of  
 372 80% of their length. The radius of each dark grey circle is proportional to the number of detected genes.  
 373 AAI values were estimated with the web-based KostasLab AAI calculator using reciprocal best hits  
 374 (<http://enve-omics.ce.gatech.edu/aai/>) for each pair of genomes. The radius of each light grey circle  
 375 is proportional to the AAI value. All data were organized according to the phylogeny given in Figure 3A.

376

377 *Some additional metabolic features and details*

378

379 We previously showed evidence that strain BW-1<sup>T</sup> relies on sulfate-reduction for  
380 growth [30]. The genome of strain BW-1<sup>T</sup> contains several sets of genes that encode the  
381 major metabolic components for respiration and reduction of sulfate. We identified a  
382 number of these genes that encode key enzymes of this pathway including two sulfate  
383 adenylyltransferases (MTBBW1\_1630032 and MTBBW1\_1740012), a set of  
384 adenylylsulfate reductases (*aprAB*) (MTBBW1\_350011 and MTBBW1\_350012), a set of  
385 sulfite reductases (*dsvABD*) (MTBBW1\_1940076, MTBBW1\_1940077,  
386 MTBBW1\_1940078 and MTBBW1\_2280030), adenosyl phosphosulfate and quinone-  
387 interacting membrane-bound oxidoreductase (*qmoABC*) (MTBBW1\_350014,  
388 MTBBW1\_350015 and MTBBW1\_350016).

389 Almost all cultured magnetotactic bacteria tested possess the capability of N<sub>2</sub>  
390 fixation [8] and strain BW-1<sup>T</sup> appears to be no exception as its genome contains a cluster  
391 of *nif* genes (*nifHEKBDJ*) demonstrating it clearly has the potential to perform this  
392 environmentally important process.

### 393 *Chemotaxonomy*

394 The cellular fatty acids of strain BW-1<sup>T</sup> were C<sub>16:1</sub> ω7c (25.2% of the total fatty  
395 acids), C<sub>16:0</sub> (23.3%), C<sub>17:1</sub> ω6c (13.1%), C<sub>15:0</sub> (9.7%), C<sub>17:0</sub> (4.9%), C<sub>15:1</sub> ω6c (4.6%),  
396 C<sub>18:1</sub> ω7c (4.4%), C<sub>17:1</sub> ω8c (3.5%), C<sub>14:0</sub> (3.2%), C<sub>18:1</sub> ω9c (1.2%). Strain BW-1<sup>T</sup> has a  
397 fatty acid profile typical of members of the *Desulfobacteraceae*, where C<sub>16:0</sub> and C<sub>16:1</sub>  
398 are generally the major fatty acids (Table 2).

399

400 **Table 2.** Major cellular fatty acids of *Desulfamplus magnetovallimortis* strain BW-1<sup>T</sup> and  
401 some phylogenetically related species.

402 Strains: *Desulfamplus magnetovallimortis* gen. nov., sp. nov. BW-1<sup>T</sup>; *Desulfobacterium autotrophicum*  
403 strain HRM2 (data from [26]); *Desulfotignum balticum* strain Sax<sup>T</sup> [26]; *Desulfospira joergensenii* strain  
404 B331<sup>T</sup> [18]; *Desulfobacter postgatei* strain DSM 2034<sup>T</sup> [26]; *Desulfobacula toluolica* strain DSM 7467<sup>T</sup>  
405 [26].

Fatty acid	BW-1 <sup>T</sup>	<i>Desulfobacterium autotrophicum</i>	<i>Desulfotignum balticum</i>	<i>Desulfospira joergensenii</i>	<i>Desulfobacter postgatei</i>	<i>Desulfobacula toluolica</i>
C <sub>14:0</sub>	3.2	2.8	3.7	13.9	15.4	8.3
iso-C <sub>15:0</sub>	-	-	-	1.8	0.7	-
C <sub>15:1</sub> ω <sub>9</sub> c	-	6.5	0.8	-	-	-
C <sub>15:0</sub>	9.7	5.1	0.2	1.3	3.1	1.6
3-OH C <sub>14:0</sub>	2.2	-	-	1.6	-	-
C <sub>16:1</sub> ω <sub>7</sub> c	25.2	-	-	-	-	-
C <sub>16:1</sub> ω <sub>9</sub> c	-	30.7	8.5	38.9	10.0	19.7
C <sub>16:0</sub>	23.3	13.2	24.3	28.4	20.9	31.2
C <sub>16:0</sub> 10-methyl	-	7.4	14.0	-	11.2	17.6
C <sub>17:1</sub> ω <sub>11</sub> c	-	10.5	-	-	-	-
C <sub>17:1</sub> ω <sub>8</sub> c	3.5	-	-	-	-	-
C <sub>17:1</sub> ω <sub>6</sub> c	13.1	-	-	-	-	-
C <sub>17:0</sub> cyclo	-	-	19.5	2.4	31.5	2.6
C <sub>17:0</sub>	4.9	2.9	0.4	0.6	2.3	0.7
C <sub>16:0</sub> 3-OH	-	0.5	-	2.3	-	-
C <sub>18:1</sub> ω <sub>13</sub> c	-	-	-	-	-	-
C <sub>18:1</sub> ω <sub>11</sub> c	-	2.7	7.4	5.3	1.2	6.0
C <sub>18:1</sub> ω <sub>9</sub> c	1.2	-	3.7	-	-	-
C <sub>18:1</sub> ω <sub>7</sub> c	4.4	-	-	-	-	-
C <sub>18:0</sub>	0.4	1.1	9.8	0.7	0.5	3.1
iso-C <sub>19:0</sub>	-	-	1.7	-	-	2.5
C <sub>19:0</sub> cyclo	-	-	1.1	-	-	-

407

408 Respiratory quinones analysis of strain BW-1<sup>T</sup> shows the presence of MK-6 (5%)  
409 and MK-7 (95%). This result is consistent with the respiratory quinones usually observed  
410 in species of the *Desulfocateraceae* family, where the menaquinone MK-7 is generally  
411 found as a major respiratory quinone.

412

413 Based on the data presented here, strain BW-1<sup>T</sup> is considered to represent a novel  
414 species of a new genus, for which the name *Desulfamplus magnetovallimortis* gen. nov.,  
415 sp. nov. is proposed.

416

417 **Description of *Desulfamplus* gen. nov.**

418 *Desulfamplus* (De.sul.fo.am'plus. N.L. prep. *de* from; N.L. pref. *sulfo-* used for  
419 N.L. sulfuratis sulfate; L.adj. *amplus*, -a, -um, large; N.L.n. masc. a large sulfate-  
420 reducer).

421 Cells are Gram-negative, relatively large rods and motile by mean of a single  
422 polar flagellum. They are strict anaerobes, using sulfate as terminal electron acceptor that  
423 is reduced to sulfide. The two main cellular fatty acids are C<sub>16:1</sub> ω7c and C<sub>16:0</sub> while the  
424 major respiratory quinone is the menaquinone MK-7. The genus *Desulfamplus* belongs to  
425 the *Deltaproteobacteria* class in the *Proteobacteria* phylum with its closest relative  
426 species belonging to genus *Desulfobacterium*. The type species is *Desulfamplus*  
427 *magnetovallimortis* BW-1<sup>T</sup>. The Taxonumber is GA00026.

428

#### 429 **Description of *Desulfamplus magnetovallimortis* sp. nov.**

430 *Desulfamplus magnetovallimortis* (ma.gne.to.val.li.mor'tis; ; L.adj.of greek origin  
431 *magnes* magnetic; L.n.fem. *valles*, -is valley; L.n.fem. *mors*, *mortis* death;  
432 *magnetovallimortis* referring to a magnetic bacterium isolated from the Death Valley in  
433 California, USA, where the type strain was isolated from).

434 Species description is as for genus, with the following additional characteristics.  
435 Cells are, on average, 2 μm long and 1 μm wide. Capable of chemoorganoheterotrophic  
436 growth, using fumarate, lactate, pyruvate, malate and succinate as electron donors and  
437 carbon sources. Sulfate and thiosulfate are used as terminal electron acceptors. Addition  
438 of at least 10 g NaCl L<sup>-1</sup> is necessary for growth; optimum NaCl concentration for growth  
439 is 15-25 g L<sup>-1</sup>. Temperature requirements: T<sub>min</sub>, 10°C; T<sub>opt</sub>, 28°C; T<sub>max</sub>, 35 °C. The pH  
440 range for growth is 6.5-7.5; optimum 7. The G-C content of the genome is 41 mol%. The  
441 major cellular fatty acids are C<sub>16:1</sub> ω7c, C<sub>16:0</sub>, C<sub>17:1</sub> ω6c, C<sub>15:0</sub>, C<sub>17:0</sub>, C<sub>15:1</sub> ω6c, C<sub>18:1</sub> ω7c,  
442 C<sub>17:1</sub> ω8c and C<sub>14:0</sub>. The type strain, BW-1<sup>T</sup> (=JCM 18010<sup>T</sup>=DSM 103535<sup>T</sup>) was isolated  
443 from a brackish spring in Death Valley National Park (California, USA). The  
444 Taxonumber is TA00086.

445

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451

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