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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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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ABSTRACT

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

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

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 ($\text{Fe(II)Fe(III)}_2\text{O}_4$)) or greigite (an iron sulfide
48 ($\text{Fe(II)Fe(III)}_2\text{S}_4$)) 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*

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

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

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

Materials and methods

Sample collection, enrichment and purification

Strain BW-1^T was isolated from mud and water collected from the brackish spring located at Badwater Basin in Death Valley, California, USA [30]. For isolation of strain BW-1, cells of magnetotactic bacteria were first magnetically concentrated from water samples using the magnetic capillary racetrack technique [56] and then inoculated into a modified semisolid, oxygen concentration gradient ([O₂]-gradient) medium based on a growth medium originally designed for *Desulfobacterium vacuolatum*. The medium consisted of an artificial seawater (ASW) base diluted to the approximate salinity of the water sample, containing (per liter): NaCl, 20 g; MgCl₂•6H₂O, 3 g; Na₂SO₄, 3 g; KCl, 0.5 g; and CaCl₂•2H₂O, 0.2 g. To this was added (per liter) the following, in order, prior to autoclaving: 50 µL of 0.2% (wt/vol) aqueous resazurin, 5 mL of modified Wolfe's mineral elixir [19], 0.3 g of NH₄Cl, 2.4 g of HEPES, 2 g of Casamino acids and 2 g of Agar Noble (both from Difco). The pH of the medium was then adjusted to 7.0 prior to autoclaving. Once the medium cooled to about 45°C, the following solutions were added (per liter), in order, from stock solutions (except for the cysteine, which was made fresh and filter sterilized directly into the medium): 0.5 mL of vitamin solution [19]; 1.8 mL of 0.5 M KHPO₄/KH₂PO₄ buffer, pH 7.0; 3 mL of 0.01 M FeCl₂ dissolved in 0.02 N HCl; and L-cysteine to give a final concentration of 0.4 g/L. The medium (10 mL) was dispensed into sterile, 15 x 125 mm screw-capped test tubes. All cultures were incubated at 28°C and, after approximately one week, an aggregate of cells formed in the anaerobic zone of the medium. The large rod-shaped cells were not magnetotactic in this medium and it was only when they were transferred in a specific liquid medium that cells became magnetotactic. An axenic culture of strain BW-1^T was obtained after three successive rounds of dilution to extinction in this latter medium. Purity of the cultures was determined using light microscopy and by amplification and sequencing of the 16S rRNA gene.

Media and culture conditions

For magnetosome formation, cells of strain BW-1^T were grown in BWM medium in 250 mL Schott bottles containing 100 mL of liquid anaerobic medium closed by a cap and a rubber stopper. The formula of the medium was (per liter): 20 g NaCl, 3 g MgCl₂•6H₂O, 3 g Na₂SO₄, 0.2 g CaCl₂•2H₂O and 0.5 g KCl. To a liter of the basal

medium, 5 ml modified Wolfe's mineral elixir [19], 50 μ L 1% aqueous resazurin, 0.3 g NH_4Cl , 2.4 g 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 1 g fumaric acid were added and the pH adjusted to 7.5 with 10 M NaOH. The medium was then autoclaved. After autoclaving, 0.5 mL of an anaerobic stock solution of vitamins [19]; 1.8 mL of 0.5 M KHPO_4 buffer (pH 7.0); 2 mL or 10 mL of 10 mM $\text{FeCL}_2 \cdot 4\text{H}_2\text{O}$ (in 0.02 M HCl) and 0.4 g of freshly made filtered sterilized cysteine were added to the medium. The medium was then bubbled with O_2 -free N_2 for 30 min. The medium was inoculated with 5 mL of a culture in exponential growth phase and incubated at 22°C.

Electron donors and acceptors were tested in this growth medium. The following compounds were tested as terminal electron acceptors (sodium salts, when appropriate): nitrate (2 mM), nitrite (2 mM), fumarate (20 mM), trimethylamine oxide (TMAO; 15 mM), dimethylsulfoxide (DMSO; 15 mM), sulfur (5 mM), sulfite (5 mM), thiosulfate (5 mM), tetrionate (5 mM), dithionite (5 mM) and N_2O (1 atm in the headspace). Except for the tubes containing nitrous oxide (N_2O), all others were flushed with O_2 -free N_2 . To test for heterotrophic growth, potential carbon sources were added to tubes to give a final concentration of 0.1% (wt/vol or vol/vol) using the BWM liquid medium minus fumaric acid. The following carbon sources were tested (sodium salts for acids, L-enantiomers were used for amino acids, D-enantiomers for sugars): alanine, aspartic acid, glutamic acid, glutamine, glycine, isoleucine, leucine, proline, serine, valine, butanol, glucose, casamino acids, yeast extract, butyrate, malate, pyruvate, acetate, fumarate, gluconic acid, lactic acid and succinate. To test for autotrophic growth, fumaric acid was omitted from the medium, 2.8 mL of a solution of 0.8 M NaHCO_3 (autoclaved dry; sterile water added after autoclaving to make the fresh stock solution) was added to the medium which was then bubbled with H_2 for 30 min. If growth was observed, the culture was transferred twice further in the same medium in triplicate. Fumaric acid was used as the positive control and sterile H_2O was used as the negative control. Tubes were incubated for at least 3 weeks at 28°C.

Whole genome sequencing and annotation

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

Phylogenetic analysis

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

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

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

Chemotaxonomic analyzes

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

Electron microscopy

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

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

Results and discussion

Cell morphology

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

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

Growth characteristics

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

Table 1. Selected characteristics that differentiate strain BW-1^T from its closest relatives.

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

Characteristic	BW-1 ^T	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 ⁻¹)	15-25	20	20	12-20	20-30	5
Optimum temperature (°C)	28	25-28	28	28-32	26-30	14-16
Compounds used as electron donor and carbon source:						
H ₂ + CO ₂	-	+	-	(+)	+	+
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^T grew anaerobically in the presence of sulfate and
261 thiosulfate, but not with nitrate, nitrite, fumarate, TMAO, DMSO, sulfite, sulfur,
262 tetrathionate, dithionite and N₂O as terminal electron acceptors.

263 Because some magnetotactic strains of *Desulfovibrio* were recently shown to
264 respire with O₂ [29], we tested BW-1^T for this ability in an [O₂]-gradient medium where
265 fumaric acid was added as the electron donor. In presence of sulfate, cells of strain BW-
266 1^T only grew as aggregates below the oxic-anoxic interface. In absence of sulfate, no
267 growth was observed showing their inability to use O₂ as electron acceptor. Strain BW-1^T
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^T 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^T also has in its genome *cydAB* genes coding for a cytochrome d ubiquinol oxidase

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

Glutamate, glutamine, casamino acids, yeast extract, fumarate, lactate, malate, pyruvate and succinate supported growth and are thus potential carbon sources for BW-1^T (Table 1).

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

Magnetosome biomineralization

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

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

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

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

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

324 *Phylogeny of strain BW-1^T*

325 Phylogenomics analyses confirmed the placement of strain BW-1^T 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^T, for which a whole
330 genome was sequenced and available, is *Desulfobacterium autotrophicum* HRM2
331 (=DSM 3382^T). 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^T and
336 relatives confirmed that the most recent ancestor of BW-1^T is shared with
337 *Desulfobacterium* species (Fig. 3B). Together with this phylogenetic evidence, ecological
338 and phenotypic features of BW-1^T compared to those of its most closely related species
339 support our contention that BW-1^T 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^T 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^T
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

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

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

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

Some additional metabolic features and details

We previously showed evidence that strain BW-1^T relies on sulfate-reduction for growth [30]. The genome of strain BW-1^T contains several sets of genes that encode the major metabolic components for respiration and reduction of sulfate. We identified a number of these genes that encode key enzymes of this pathway including two sulfate adenylyltransferases (MTBBW1_1630032 and MTBBW1_1740012), a set of adenylylsulfate reductases (*aprAB*) (MTBBW1_350011 and MTBBW1_350012), a set of sulfite reductases (*dsvABD*) (MTBBW1_1940076, MTBBW1_1940077, MTBBW1_1940078 and MTBBW1_2280030), adenosyl phosphosulfate and quinone-interacting membrane-bound oxidoreductase (*qmoABC*) (MTBBW1_350014, MTBBW1_350015 and MTBBW1_350016).

Almost all cultured magnetotactic bacteria tested possess the capability of N₂ fixation [8] and strain BW-1^T appears to be no exception as its genome contains a cluster of *nif* genes (*nifHEKBDJ*) demonstrating it clearly has the potential to perform this environmentally important process.

Chemotaxonomy

The cellular fatty acids of strain BW-1^T were C_{16:1} ω7c (25.2% of the total fatty acids), C_{16:0} (23.3%), C_{17:1} ω6c (13.1%), C_{15:0} (9.7%), C_{17:0} (4.9%), C_{15:1} ω6c (4.6%), C_{18:1} ω7c (4.4%), C_{17:1} ω8c (3.5%), C_{14:0} (3.2%), C_{18:1} ω9c (1.2%). Strain BW-1^T has a fatty acid profile typical of members of the *Desulfobacteraceae*, where C_{16:0} and C_{16:1} are generally the major fatty acids (Table 2).

Table 2. Major cellular fatty acids of *Desulfamplus magnetovallimortis* strain BW-1^T and some phylogenetically related species.

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

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

407

408 Respiratory quinones analysis of strain BW-1^T 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^T 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.**

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

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

Description of *Desulfamplus magnetovallimortis* sp. nov.

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

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

445

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