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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 *D. magnetovallimortis* is BW-1^T (JCM 18010^T–DSM 103535^T).

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Introduction

Magnetotactic bacteria represent a phylogenetically, ecologically and metabolically diverse group of prokaryotes able to biomineralize single-magnetic-domain nanocrystals of magnetite (an iron oxide ($Fe(II)Fe(III)_2O_4$)) or greigite (an iron sulfide ($Fe(II)Fe(III)_2S_4$)) in specific organelles [1]. These organelles, called magnetosomes, are aligned in one or more chains within the cytoplasm and cause cells to actively swim along the Earth's magnetic field lines [2]. This behavior, termed magnetotaxis, makes the search for optimal concentrations of certain nutrients and redox conditions within water columns and sediments more efficient by simplifying a three-dimensional search to a linear search [2].

Although magnetotactic bacteria were discovered 40 years ago [3], relatively few strains have been cultivated in pure culture. Officially described and cultured magnetotactic species are all magnetite-producers and belong to the genera *Magnetospirillum* [4–6], *Desulfovibrio* [7], *Magnetovibrio* [8], *Magnetococcus* [9] and *Magnetospira* [10]. The candidate genus name of Magnetoglobus has also been officially proposed for a magnetotactic multicellular prokaryote [11].

Greigite-producing magnetotactic bacteria were first described in 1983 [12] but the mineral composition of the crystals in the magnetosomes of these organisms was only identified in 1990 [13,14]. Until recently, they were thought to be confined to marine habitats [15]. There are two known morphological types of greigite-producers: a group of morphologically unique, multicellular magnetotactic prokaryotes (MMPs), and a group of large rod-shaped bacteria [16]. While a good deal of information has been acquired regarding the MMPs from culture-independent,

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environmental [17–21] and genomics studies [22–24], their isolation in axenic culture has not yet been successful, although *Candidatus* Magnetoglobus multicellularis was co-cultured in a medium where cells reduced sulfate and used succinate as an electron donor [23]. The MMP group represents an assemblage of obligately multicellular organisms that consist of about 10–60 genetically identical cells and is phylogenetically affiliated with the *Desulfobacteraceae* family within the *Deltaproteobacteria* class of the *Proteobacteria* phylum [25,26,19–21,27]. The large rod-shaped greigite and/or magnetite-producing bacteria have been studied for several decades using culture-independent techniques from environmental samples [28–31,15] but have only been phylogenetically identified [32,33] and cultured [32] recently. The greigite-producing, rod-shaped bacteria also belong to the *Desulfobacteraceae* family [32].

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 [32]. *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 [32,34]. 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 [24]. Magnetosome genes involved in greigite formation were first described in *Ca.* Magnetoglobus multicellularis [22]. Finally, greigite magnetosome genes were also identified in a draft genome of an uncultivated bacterium belonging to the candidate phylum *Latescibacteria* [35]. 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*.

D. 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 [32]. 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 [32]. For isolation of strain BW-1, cells of magnetotactic bacteria were first magnetically concentrated from water samples using the magnetic capillary racetrack technique [36] and then inoculated into a modified semisolid, oxygen concentration gradient ([O_2]-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; $MgCl_2 \cdot 6H_2O$, 3 g; Na_2SO_4, 3 g; KCl, 0.5 g; and $CaCl_2 \cdot 2H_2O$, 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 [2], 0.3 g of NH_4Cl, 2.4 g of HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 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 [2]; 1.8 mL of 0.5 M $KHPO_4$/$KHPO_4$ buffer, pH 7.0; 3 mL of 0.01 M $FeCl_2$ 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 × 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 $MgCl_2 \cdot 6H_2O$, 3 g Na_2SO_4, 0.2 g $CaCl_2 \cdot 2H_2O$ and 0.5 g KCl. To a liter of the basal medium, 5 ml modified Wolfe's mineral elixir [2], 50 μL 1% aqueous resazurin, 0.3 g NH_4Cl, 2.4 g 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 [2]; 1.8 mL of 0.5 M $KHPO_4$ buffer (pH 7.0); 2 mL or 10 mL of 10 mM $FeCL_2 \cdot 4H_2O$ (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), tetrationate (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 *D. magnetovallimortis* strain BW-1 was previously described [37]. 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

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