

Magnetospirillum bellicus sp. nov., a Novel Dissimilatory Perchlorate-Reducing Alphaproteobacterium Isolated from a Bioelectrical Reactor[∇]

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Previously isolated dissimilatory perchlorate-reducing bacteria (DPRB) have been primarily affiliated with the *Betaproteobacteria*. Enrichments from the cathodic chamber of a bioelectrical reactor (BER) inoculated from creek water in Berkeley, CA, yielded a novel organism most closely related to a previously described strain, WD (99% 16S rRNA gene identity). Strain VDY^T has 96% 16S rRNA gene identity to both *Magnetospirillum gryphiswaldense* and *Magnetospirillum magnetotacticum*, and along with strain WD, distinguishes a clade of perchlorate-reducing *Magnetospirillum* species in the *Alphaproteobacteria*. In spite of the phylogenetic location of VDY^T, attempted PCR for the key magnetosome formation genes *mamI* and *mamL* was negative. Strain VDY^T was motile, non-spore forming, and, in addition to perchlorate, could use oxygen, chlorate, nitrate, nitrite, and nitrous oxide as alternative electron acceptors with acetate as the electron donor. Transient chlorate accumulation occurred during respiration of perchlorate. The organism made use of fermentation end products, such as acetate and ethanol, as carbon sources and electron donors for heterotrophic growth, and in addition, strain VDY^T could grow chemolithotrophically with hydrogen serving as the electron donor. VDY^T contains a copy of the RuBisCo *cbbM* gene, which was expressed under autotrophic but not heterotrophic conditions. DNA-DNA hybridization with strain WD confirmed VDY^T as a separate species (46.2% identity), and the name *Magnetospirillum bellicus* sp. nov. (DSM 21662, ATCC BAA-1730) is proposed.

Dissimilatory perchlorate-reducing bacteria (DPRB) use perchlorate as a terminal electron acceptor during respiration, reducing it completely to chloride. As a consequence, bioremediation of perchlorate has been identified as the most effective means of treating this harmful contaminant (10), which, due to historically unregulated release into the environment, has become widespread (13, 20, 41). Fortunately, DPRB are ubiquitous and can be readily isolated from a variety of environments (1, 10, 11, 39, 44), and a key gene in the pathway, the chlorite dismutase (*cld*) gene, has been broadly detected (6). Much has been revealed about the biochemistry and genetics of microbial perchlorate reduction through the study of several model organisms, including *Dechloromonas aromatica* and *Dechloromonas agitata*, by a variety of groups (5, 6, 8, 9, 17, 28, 29, 34, 35, 38, 47, 51, 56, 57).

Less is known about the variation in physiology between these organisms or the evolution of the perchlorate reduction metabolism, highlighting a need for further isolation and characterization of pure cultures. The lack of congruence between phylogenetic trees of *cld* and the 16S rRNA gene among tested DPRB suggests that the metabolism may be the result of horizontal gene transfer (6). Given that various elements of the pathway may be mobile, it is not unreasonable to expect that organisms with a wide phylogenetic diversity could acquire the ability to reduce perchlorate. As more varied enrichment con-

ditions are tested (2, 39), sometimes as a result of novel bio-reactor development for perchlorate treatment (38, 40, 45), the true phylogenetic diversity of DPRB is becoming apparent, supporting the hypothesis that the metabolism may be widespread within the tree of life, similar to other respiratory processes, such as the reduction of sulfate, Fe(III), and nitrate.

Although perchlorate has been primarily regarded as an anthropogenic contaminant, a variety of studies have indicated that perchlorate occurs naturally (29–31, 34), which provides a possible explanation for the selective pressure behind the evolution of perchlorate reduction genes. As more is understood about the chlorine redox cycle on earth, knowledge about the diversity of organisms capable of interacting with the various oxyanions of chlorine is becoming more important. Here, we report the characterization of a unique DPRB in the *Alphaproteobacteria*. Strain VDY^T was isolated from the surface of a working electrode in an active perchlorate-reducing bioelectrical reactor (BER) that was inoculated with water from Strawberry Creek on the University of California, Berkeley, campus (40). This is only the second described DPRB in the *Alphaproteobacteria*, the other being the closely related strain WD (26), and these strains compose a unique clade of perchlorate-reducing organisms in the genus *Magnetospirillum*.

MATERIALS AND METHODS

Media and culturing conditions. Culturing medium was prepared as described previously (8). All cultures were grown and evaluated using freshwater 30 mM bicarbonate-buffered basal medium (pH 6.8) under an N₂-CO₂ (80%/20%) headspace, except for testing the optimum pH for growth, where a 10 mM phosphate-buffered medium was utilized. NaH₂PO₄ and Na₂HPO₄ were added in appropriate concentrations to establish stable pHs at 6.0, 6.5, 6.8, 7.0, 7.2, and 7.5. The medium was boiled, cooled, and dispensed under an N₂ headspace. Omitting

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TABLE 1. Primers used in this study

Primer name	Gene target	Sequence
5EcoI	<i>mamI</i>	GGCGAATTCATGCCAAGCGT GATTTTCGG
3SpeI	<i>mamI</i>	GGCACTAGTTCAACCATCGA TGTCAGGG
5EcoL	<i>mamL</i>	GGCGAATTCATGGTAAGATT GATCGGATC
3SpeL	<i>mamL</i>	GGACTAGTTCAGCGCTTGAT GACGATG
595f	RuBisCo <i>cbbl</i>	GACTTCACCAAAGACGACGA
1387r	RuBisCo <i>cbbl</i>	TCGAACTTGATTTCTTTCCA
cbbMf	RuBisCo <i>cbblM</i>	TCATCAARCCSAARCTSGGCC TGCGTCCC
cbbMr	RuBisCo <i>cbblM</i>	MGAGGTGACSGCRCCGTGR CCRGCMCGRTG

sodium bicarbonate, all other medium components were identical with bicarbonate-buffered medium. Electron acceptors and donors were added separately from sterile, anoxic aqueous stock solutions. For testing of salinity growth optima, NaCl was added to the culture medium from a 5 M sterile, anoxic aqueous stock solution. Acetate and perchlorate (10 mM) were used for testing of alternative electron donors and acceptors, respectively, and together for testing optimum growth temperature, pH, and salinity.

Scanning electron microscopy (SEM). Cells for electron microscopy were grown anaerobically in freshwater basal medium amended with acetate (10 mM) and perchlorate (10 mM) and prepared and imaged as described previously (42).

16S rRNA gene sequencing and analysis. Isolation of genomic DNA, 16S rRNA gene-specific PCR, and sequencing of PCR products were completed as described previously (11, 40). 16S rRNA gene sequences were aligned with Muscle 3.6 (14), and Bayesian analysis of 16S rRNA gene phylogeny was completed with MrBayes 3.2 (19, 33). The program was run until the standard deviation of the split frequencies was stabilized below 0.01, in this case for 241,000 generations, with a sample frequency of 1,000. The first 25% of the samples were discarded for accurate estimation of the posterior probability distribution of the summary tree. The organisms in the *Acetobacteraceae* that were used were *Acetobacter pasteurianus*^T (X71863), *Acetobacter cerevisiae*^T (AJ419843), *Gluconobacter oxydans*^T (X73820), *Gluconobacter cerinus*^T (X80775), *Paracraurococcus ruber*^T (D85827), *Gluconacetobacter oboediens*^T (AJ001631), *Gluconacetobacter europaeus*^T (Z21936), *Gluconacetobacter hansenii*^T (X75620), *Roseococcus thiosulfatophilus*^T (X72908), *Acidomonas methanolica*^T (D30770), *Acidocella facilis*^T (D30774), *Acidiphilium aminolytica*^T (D30771), and *Acidiphilium cryptum*^T (D30773); the organisms in the *Rickettsiales* that were used were *Rickettsia honei*^T (AF060705), *Rickettsia australis*^T (L36101), *Rickettsia aeschlimannii*^T (U74757), *Wolbachia pipientis* (AF179630), and *Holospora obtusa* (X58198). For all other accession numbers, see Fig. 2.

DNA analysis. DNA-DNA hybridization was performed commercially at the DSMZ. DNA was purified and hybridized as described previously (10, 16, 27). The G+C content was determined by high-performance liquid chromatography (HPLC) at the DSMZ as previously outlined (25).

Fatty acid determination. Cells were grown in freshwater basal medium, harvested by centrifugation, and resuspended in 1 ml buffer. The whole-cell fatty acid content was determined using the Sherlock Microbial Identification System (v. 4.5; MIDI, Newark, DE) according to the manufacturer's protocol.

***mamI/mamL* PCR.** Genomic DNA was isolated using the Power Soil Kit (MoBio) as directed by the manufacturer's protocol. Primers specific to *mamI* and *mamL* (27) were used to amplify these genes (Table 1). The following PCR parameters were used: 95°C for 5 min, then 32 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a final incubation at 72°C for 10 min. Reaction mixtures (50 µl) contained 3 µl (100 ng) genomic DNA as the template, 1 µl (20 µM) each primer, and 1.25 U *Taq* polymerase (TaKaRa). Genomic DNA from *Magnetospirillum magneticum* AMB-1 was used as the positive control.

RNA isolation. Cultures were grown in freshwater basal medium with an N₂-CO₂ (80%/20%) headspace under autotrophic (H₂ as the sole electron donor; CO₂ as the carbon source) and heterotrophic (acetate as the electron donor and carbon source) conditions with perchlorate as the sole terminal electron acceptor. Total RNA was isolated by trapping cells on a nylon membrane filter

(Nalgene) via vacuum filtration, immersing the filter in 1 ml Trizol reagent (Sigma-Aldrich) in Lysing Matrix E (MP Bio) tubes, and bead beating for 30 s. After a 5-min room temperature incubation, 200 µl chloroform (Fisher Scientific) was added, and the tubes were shaken for 30 s and allowed to incubate at room temperature for a further 10 min. The tubes were then centrifuged (13,000 rpm) at 4°C for 15 min, and the supernatant was transferred to new tubes to which 500 µl diethyl pyrocarbonate (DEPC)-H₂O was added. The tubes were mixed, supplemented with 1 ml isopropanol (Sigma-Aldrich), mixed again, and allowed to incubate at room temperature for 10 min. The tubes were then centrifuged (13,000 rpm) at 4°C for 15 min, the supernatant was decanted and replaced with 1 ml 75% ethanol, and the tubes were vortexed and centrifuged (13,000 rpm) again at 4°C for 5 min. After the supernatant was decanted, the pellets were allowed to air dry for 10 min and resuspended in 100 µl DEPC-H₂O. The resuspended nucleic acids were then further purified using the AllPrep DNA/RNA Mini Kit (Qiagen) according to the manufacturer's protocol, including the optional DNase I steps (E1 to E4).

cDNA preparation. Total RNA (7 µl) from the isolation protocol was added to 1 µl of 3 µM *cbblM*-specific primers (15, 43) (Table 1), and 4 µl 2.5 mM each deoxynucleoside triphosphate (dNTP) (Invitrogen); heated at 65°C for 5 min; and quickly chilled on ice. To this, 4 µl of 5× First-Strand buffer (Invitrogen), 2 µl 0.1 M dithiothreitol (DTT) (Invitrogen), and 1 µl RNaseOUT (Invitrogen) were added, mixed gently, and incubated at 42°C for 2 min. Superscript II Reverse Transcriptase (0.5 µl; Invitrogen) was added, mixed gently, and incubated at 42°C for 50 min and then again at 70°C for 15 min.

RuBisCo *cbblM* and *cbbl* PCR. Primers designed to amplify form I (*cbbl*) and form II (*cbblM*) of the RuBisCo large subunit were utilized as previously described (15, 43) (Table 1). Amplifications involved the following parameters: 95°C for 4 min, then 35 cycles of 95°C for 45 s, 57°C for 45 s, and 72°C for 45 s, followed by a final incubation at 72°C for 10 min. The PCR products were visualized by agarose gel electrophoresis and imaged using a NucleoTech gel-imaging system.

Analytical methods. All experimental analyses were performed in triplicate to ensure reproducibility, and the results are expressed as the mean of these determinations. The concentrations of perchlorate and chlorate in cultures were determined using ion chromatography as previously described (9). Cell growth in active cultures was monitored by the optical density at 600 nm and by the total direct cell count using phase-contrast microscopy (Petroff-Hausser counting chamber; 0.02-mm depth). Samples collected for counting were immediately fixed in 0.2 µm filter-sterilized formaldehyde (final concentration, 3.7%).

RESULTS

Phylogenetic characterization. Strain VDY^T was spirillum shaped, ~0.5 µm by ~3 µm (Fig. 1), typically with two helical rotations, though cells were observed with as few as one or as many as five, and were highly motile. Phylogenetic analysis of the 16S rRNA gene sequence indicated that it was most closely related to the previously described strain WD (99% 16S rRNA gene sequence similarity) (26) and placed it within the family *Rhodospirillaceae* of the *Alphaproteobacteria* (Fig. 2). DNA-DNA hybridization between strain VDY^T and strain WD showed 46.2% similarity, well below the 70% similarity required to distinguish the two strains as separate species. The next closest organism to strain VDY^T based on 16S rRNA gene identity was *Magnetospirillum gryphiswaldense* MSR-1, with 96% sequence similarity between the two.

Magnetosome formation. To further examine the distinction between strain VDY^T and *Magnetospirillum* species, it was tested for magnetosome production via visual inspection of response to a magnetic field and molecular probing. Strain VDY^T was unable to produce magnetosomes in *Magnetospirillum*-specific medium under microaerophilic conditions (21) or in high ferrous-iron concentrations (5 to 10 mM FeCl₂) in its typical culture medium (data not shown). *mamI* and *mamL* are genes required for magnetosome formation in *Magnetospirillum* strains AMB-1, MS-1, and MSR-1 (32). PCR amplification using AMB-1-specific primers for these genes (27) (and pre-

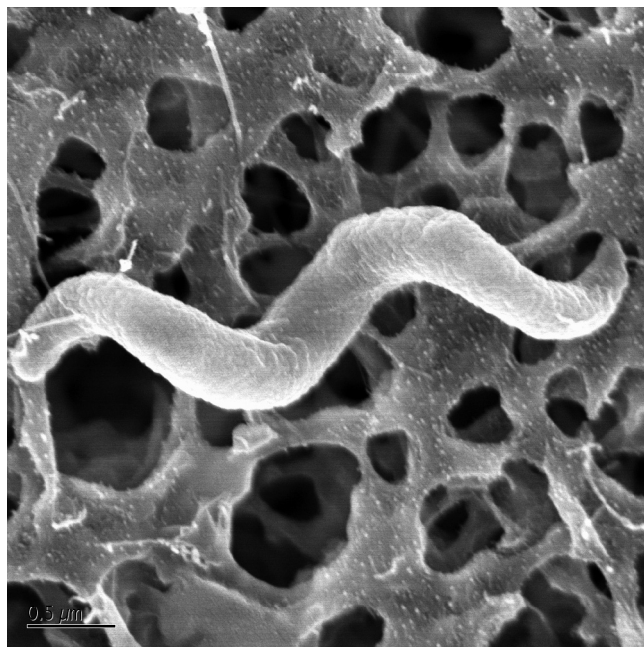


FIG. 1. SEM of strain VDY^T. Bar, 0.5 μm.

dicted by BLAST to also be specific for *M. gryphiswaldense mamI* and *mamL* genes) was negative in VDY^T compared to AMB-1-positive controls (data not shown).

Fatty acid profiles. VDY^T contained a fatty acid profile very similar to that of WD when grown on perchlorate (10 mM) and acetate (10 mM) (Table 2). The major fatty acid present for both was 18:1ω7c. Secondary fatty acids were the summed feature 16:1ω7c/15 iso 2OH and 16:0. These three fatty acids made up 87.12% and 89.80% of the fatty acid content of strains VDY^T and WD, respectively, and were the only fatty acids present above 5% of the total.

Growth characteristics. Strain VDY^T grew between 10 and 42°C, but not at 50°C, with an optimum growth rate at 42°C (0.29 h⁻¹) at pH 6.8 (Table 3). It could grow with NaCl concentrations up to 1.5%, but not at 2%, and showed minimal growth defects in medium containing up to 40 mM perchlorate or chlorate. VDY^T was non-spore forming, as indicated by the inability of cultures to grow in new medium after incubation at 80°C for 3 min, and was an obligate respiratory organism, being unable to ferment hexoses, Casamino Acids, or yeast extract components. Strain VDY^T used electron acceptors similar to those of strain WD and other known DPRB (1, 8, 26), but a wider range than the previously characterized *Magnetospirillum* species, namely, oxygen, perchlorate, chlorate, and nitrate, with the additional ability to use nitrite and nitrous oxide (Table 3). VDY^T had a higher growth rate on nitrate than on perchlorate (Fig. 3a). Like other *Magnetospirillum* species, strain VDY^T could oxidize a range of fermentation end products, including acetate, lactate, and ethanol, but no sugars (Table 3). VDY^T could also utilize the electron donors FeCl₂, H₂, and the humic substances analog anthrohydroquinone-2,6-disulfonate (AHDS). Chlorate transiently accumulated during growth on perchlorate, but after 4 h, chlorate was removed concomitantly with perchlorate (Fig. 3b).

Chemolithotrophy. Strain VDY^T could grow under both mixotrophic and autotrophic conditions with hydrogen coupled to perchlorate reduction, which has been described for only three other pure cultures (39, 45). Figure 4 shows chemolithotrophic growth of strain VDY^T with hydrogen as the electron donor and perchlorate as the sole terminal electron acceptor in the bicarbonate-buffered medium described above. Cells were grown in electron donor-limited medium (10 mM acetate and 15 mM perchlorate) and allowed to reach late stationary phase to reduce the possibility of acetate carryover. Growth was monitored by direct cell counts. Cells reduced 2.2 mM perchlorate over 48 h relative to a no-electron-donor control and increased in number from $6.5 \times 10^7 (\pm 1.2 \times 10^7)$ to $1.1 \times 10^8 (\pm 2.1 \times 10^7)$ cells · ml⁻¹ in the first 24 h (Fig. 4). The control, from which the electron donor (H₂) was omitted, showed no significant growth. Interestingly, although the cultures entered stationary phase after 24 h of incubation, the organisms continued to respire the perchlorate to completion over 48 h.

Genetic screening demonstrated the presence of the RuBisCo *cbbM* large-subunit (form II) gene (Fig. 5, lane 3), but not the *cbbL* (form I) gene (data not shown), and differential expression of *cbbM* mRNA under heterotrophic and autotrophic conditions. *cbbM* was expressed when cells were grown on hydrogen and perchlorate as the sole electron donor and acceptor, respectively, under an N₂-CO₂ (80%/20%) head-space (Fig. 5, lane 6). In contrast, no *cbbM* expression (Fig. 5, lane 4) was observed in cells grown under identical conditions with acetate as the electron donor and carbon source. Since upregulation of RuBisCo under autotrophic conditions would be expected if this gene were present and differentially expressed, these data support the involvement of RuBisCo in carbon fixation by strain VDY^T.

DISCUSSION

These studies examined the phylogeny and physiology of strain VDY^T and included molecular probing to assess the presence of magnetosome-forming genes and those responsible for autotrophic growth. PCR for the *mamL* and *mamI* genes (essential for magnetosome formation in other *Magnetospirillum* species) was negative, although this does not rule out the possibility that homologs undetected by these primers exist. However, strain VDY^T was unable to form magnetosomes in the typical medium used for testing *Magnetospirillum* species, nor was it able to form magnetosomes in other medium with ferrous iron present. The inability of an organism closely related to *Magnetospirillum* species to form magnetosomes is not unprecedented. Strain WD and four other nonmagnetotactic *Magnetospirillum* strains, including J10 and CC-26 (Table 3), have been reported (17, 26, 37), and a non-magnetosome-forming strain of *M. gryphiswaldense* was described which contained a large genomic lesion corresponding to loss of the magnetosome island (36). Similarly, it is also not unprecedented for a member of the genus *Magnetospirillum* to contain perchlorate reduction genes. *M. magnetotacticum* contains a homolog of the *cld* gene (6), but it lacks all other perchlorate reduction genes. The *cld* gene sequences of strains VDY, WD, and MS-1 are more similar to each other than those of other strains containing *cld*, but this gene is not found in other sequenced *Magnetospirillum* species (6; J. C.

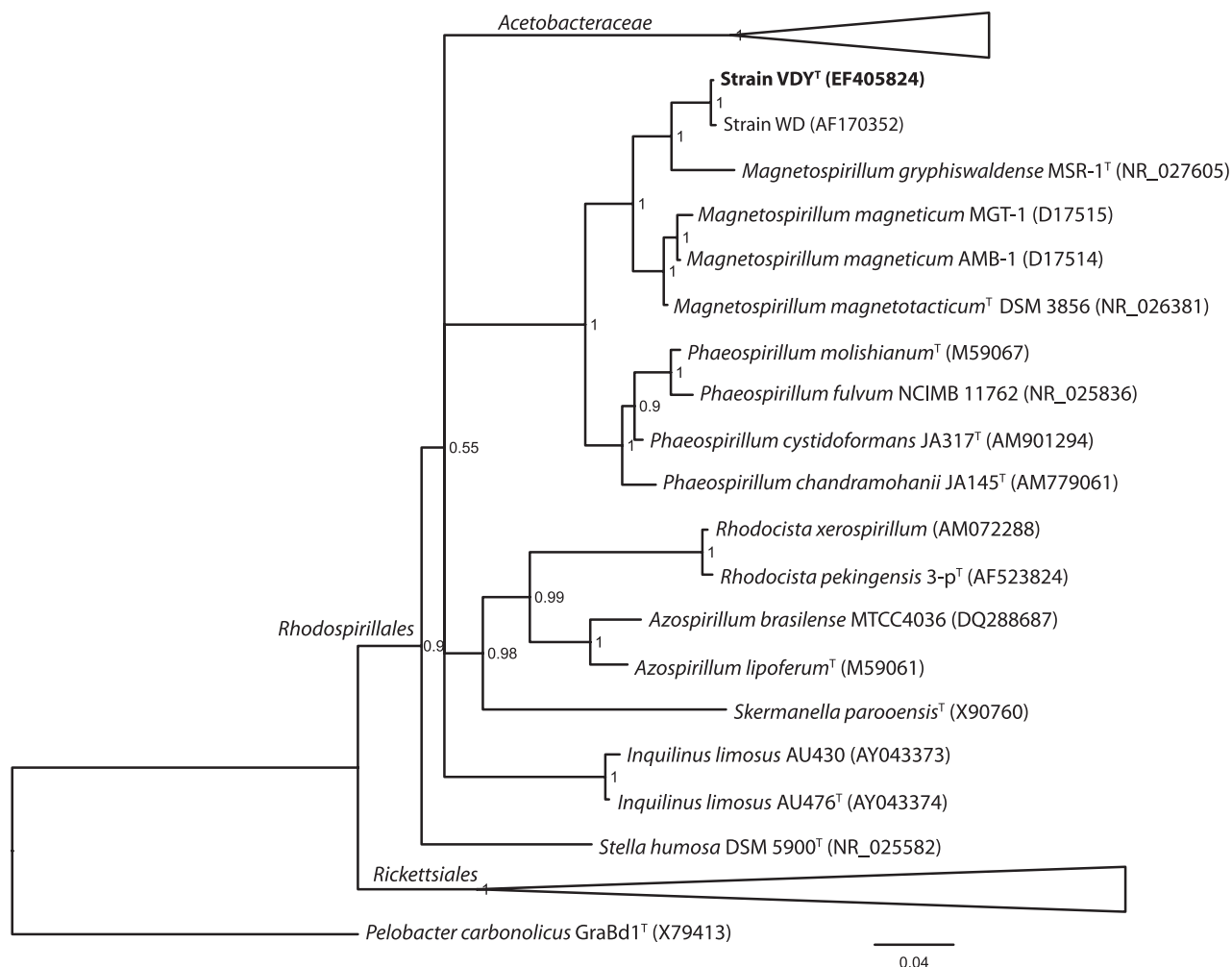


FIG. 2. Phylogenetic placement of *Dechlorospirillum* among the *Rhodospirillales* in the *Alphaproteobacteria* according to 16S rRNA gene analysis. The bar represents 0.04 expected change per site; posterior probability values are indicated at the nodes.

TABLE 2. Comparative fatty acid compositions of strains VDY^T and WD

Fatty acid type	Content ^a	
	VDY ^T	WD
12:0	3.25	3.14
14:0	0.37	0.43
16:1 ω 7c		
16:0	12.01	9.87
16:0 3OH	0.75	0.69
18:1 ω 9c		
18:1 ω 7c	64.64	68.51
18:1		
18:0	1.89	0.80
11 methyl 18:1 ω 7c	0.83	1.36
18:1 2OH	1.23	
18:0 3OH	0.33	
Summed features		
14:0 3OH/16:1 iso i	3.10	2.86
16:1 ω 7c/15 iso 2OH	10.47	11.42
18:1c11/t9/t6		
19:1 2OH/cy 19:0		
Unknown	3.59	3.28

^a Values are given as percentages of total membrane content. Boldface indicates values for the major fatty acid.

Thrash, K. Byrne-Bailey, and J. D. Coates, unpublished results). These data and those presented in this study suggest that magnetosome-related and perchlorate reduction genes are poor taxonomic markers.

VDY^T had some other notable physiological and genetic features. The temporary chlorate accumulation in strain VDY^T indicates a differentiation between the initial perchlorate reduction step in strain VDY^T and other DPRB. A recent study quantified the kinetic difference in another chlorate-accumulating DPRB, *Azospira* strain PCC (12), and found that the chlorate accumulation by strain PCC was consistent with competitive inhibition of chlorate reduction by perchlorate. Two other recently reported strains also showed similar chlorate accumulation (39), generally around 20% of the initial perchlorate concentration, whereas in strain VDY^T, the accumulation was closer to 1%. VDY^T, therefore, represents a third class of perchlorate reduction physiology intermediate between no accumulation (in strains like *D. agitata* [8]) and the considerable accumulation of chlorate seen in strains PCC, MP, and CR. Comparative analysis of the *pcr* genes and enzymes between organisms from these three classes could yield

TABLE 3. Physiological characteristics of strain VDY^T compared to those of closely related *Magnetospirillum* strains^a

Characteristic	Value ^b						
	VDY ^T	WD	<i>M. gryphiswaldense</i>	<i>M. magnetotacticum</i>	<i>M. magneticum</i>	J10	CC-26
Cell type	Spirillum	Spirillum	Spirillum	Spirillum	Spirillum	Spirillum	Spirillum
Size (μm)	~0.5 by ~3	~0.2 by ~7	0.7 by ~4	~0.4 by ~4	~0.5 by >3	~0.5 by ~4	~0.5 by 2–5
Motility	+	+	+	+	+	+	+
Temp range (°C)	<10–42	25–37	ND	15–37	ND	ND	ND
Temp optima (°C)	42	35	28–34	30	28–34	ND	ND
pH range	6.0–7.5	6.5–7.5	ND	ND	5.0–8.2	ND	ND
pH optima	6.8	7.2	7.0–7.5	7.0–7.5	7.0–7.5	ND	ND
NaCl tolerance (%)	1.5	1	ND	<1	ND	ND	ND
Spore forming	–	–	ND	ND	ND	ND	ND
Fermentative	–	–	–	–	–	ND	ND
G+C content (%)	64.8	66.1	71	66.4	65.1	ND	ND
RuBisCo <i>cbbM</i>	+	ND	+	+	+	+	ND
<i>cld</i>	+	+	–	+	–	ND	ND
Magnetotactic	–	–	+	+	+	–	–
Electron acceptors							
Oxygen	+	+	+	+	+	+	+
Perchlorate	+	+	ND	ND	ND	–	ND
Chlorate	+	+	ND	ND	ND	–	ND
Nitrate	+	+	–	+ ^c	+	+	+
Nitrite	+	ND	ND	ND	ND	ND	ND
N ₂ O	+	ND	ND	ND	ND	ND	ND
Sulfate	–	–	ND	ND	ND	–	ND
Thiosulfate	–	–	ND	ND	ND	ND	ND
Selenate	–	–	ND	ND	ND	ND	ND
Arsenate	–	ND	NA	ND	ND	ND	ND
Fumarate	–	–	ND	ND	ND	–	ND
Malate	–	–	ND	ND	ND	ND	ND
Fe(III) NTA ^d	–	–	ND	ND	ND	ND	ND
AQDS	–	–	ND	ND	ND	ND	ND
Electron donors							
Acetate	+	+	+	+	+	+	+
Propionate	+	+	ND	–	+	ND	+
Isobutyrate	+	+	ND	–	ND	ND	+
Butyrate	+	+	ND	–	+	ND	+
Valerate	+	+	ND	ND	ND	ND	–
Formate	–	–	ND	–	ND	ND	–
Methanol	–	–	ND	–	ND	ND	–
Ethanol	+	+	ND	–	ND	ND	–
Catechol	–	–	ND	–	ND	ND	–
Glycerol	–	–	ND	–	ND	ND	–
Benzoate	–	–	ND	–	ND	ND	+
Pyruvate	+	ND	+	+	+	ND	+
Citrate	–	–	ND	–	ND	ND	–
Succinate	+	+	+	+	+	ND	+
Lactate	+	+	+	+	+	ND	+
Glucose	–	–	ND	–	ND	ND	–
Sucrose	–	ND	–	ND	ND	ND	–
Fructose	–	ND	ND	–	ND	ND	–
Maltose	+	ND	–	–	ND	ND	–
Casamino Acids	+	+	ND	ND	ND	ND	ND
Fumarate	+	+	ND	+	+	ND	+
Malate	+	+	+	+	+	ND	+
Hydrogen	+	–	ND	ND	ND	ND	ND
FeCl ₂	+	+	ND	ND	ND	–	ND
AHDS	+	ND	ND	ND	ND	ND	ND
H ₂ S	–	ND	+	ND	ND	+	ND
Thiosulfate	ND	ND	+	ND	ND	+	ND
Methane	–	ND	ND	ND	ND	ND	ND
Urea	–	ND	ND	ND	ND	ND	ND

^a Data for the table were compiled from references 3, 4, 6, 7, 16–18, 22–24, 26, 35, and 37 and the IMG/M website (for G+C content of *M. magnetotacticum* and *M. magneticum*).

^b +, present or, with regard to electron donors/acceptors, positive for utilization (for VDY^T and WD, this was indicated by three successful transfers on the substrate); –, absent or not utilized; ND, not determined.

^c Nitrate was coreduced with oxygen.

^d NTA, nitrilotriacetic acid.

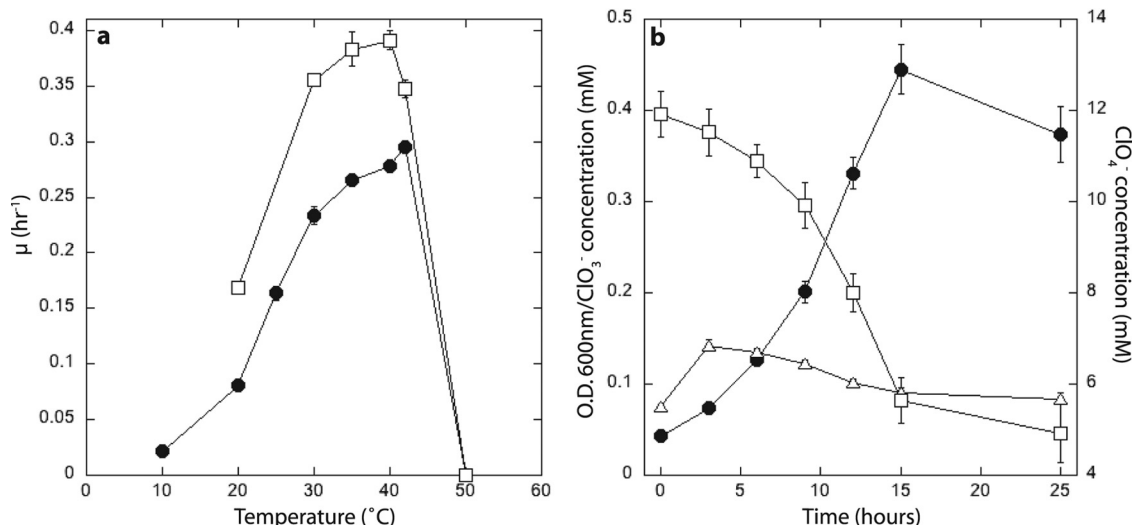


FIG. 3. (a) Comparative temperature-dependent growth optima of VDY^T on nitrate (open squares) and perchlorate (closed circles). (b) Transient chlorate accumulation by strain VDY^T while growing on perchlorate. Closed circles, optical density at 600 nm (O.D. 600 nm); open squares, ClO₄⁻; open triangles, ClO₃⁻. The error bars represent the standard deviations of triplicate samples.

important information about the evolution of the perchlorate reduction pathway. The presence and differential expression of RuBisCo is enticing evidence for this autotrophy pathway in strain VDY^T and is the first evidence for a specific mechanism of carbon fixation in DPRB. However, given that cells continued to respire after growth was completed, some form of energy storage by the organism cannot be ruled out.

Taxonomic conclusions. The taxonomic characterization of strain VDY^T has demonstrated that the strain shares many characteristics with strain WD and other *Magnetospirillum* species but is nevertheless distinct. Like all described *Magnetospi-*

rillum species, VDY^T is strictly respiratory, motile, and Gram negative and did not utilize the sugars tested. However, like strain J10, CC-26, or WD, strain VDY^T did not form magnetosomes under the conditions tested and, furthermore, does not contain copies of the necessary magnetosome formation genes *mamI* and *mamL* with close homology to those found in AMB-1. Although these results are negative data and not sufficient to prove that magnetosome formation is impossible, they add to the physiological and genetic distinction between strain VDY^T and other *Magnetospirillum* species. VDY^T uses a wide range of electron acceptors, similarly to strain WD and in contrast to other *Magnetospirillum* species. Since it is only 4% divergent at the 16S rRNA gene from *M. gryphiswaldense*, VDY^T may not be considered a novel genus, but this divergence is enough for it to be considered a novel species. The low DNA-DNA hybridization value between this organism and its closest phylogenetic relative, strain WD, also distinguishes strain VDY^T as a separate species from strain WD. Thus, based on physiological and phylogenetic characteristics, strain VDY^T should be considered a distinct species of *Magnetospirillum*.

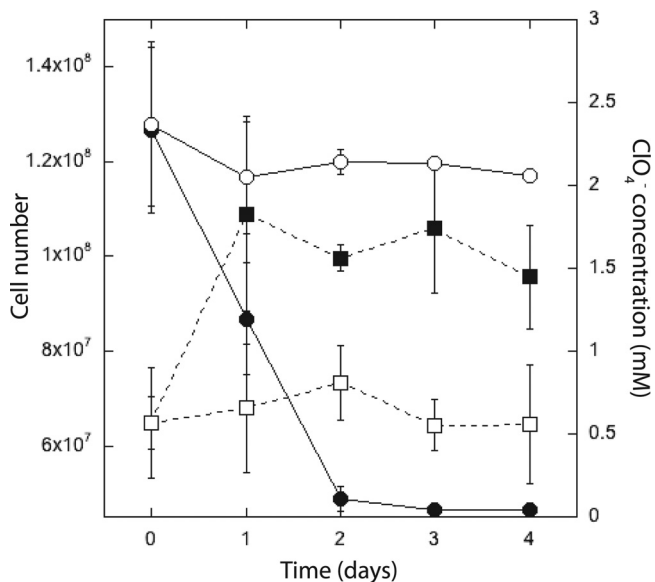


FIG. 4. Chemolithoautotrophic growth of strain VDY^T (closed squares) compared to that of a control with no added electron donor (open squares), showing concomitant perchlorate reduction (closed circles, experimental; open circles, control). The error bars represent the standard deviations of triplicate samples.

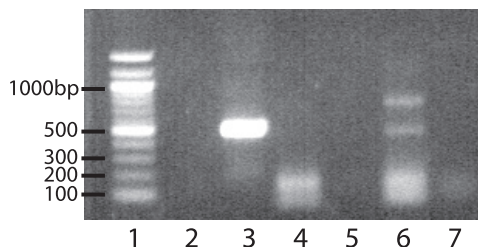


FIG. 5. Reverse transcription (RT)-PCR of the RuBisCO *cbbM* gene. Strain VDY^T contains a genomic copy of the *cbbM* gene (lane 3), that is differentially expressed during chemolithoautotrophic growth (lane 6), but not chemoorganoheterotrophic growth (lane 4). Lane 1, 100-bp ladder; lane 2, negative control (water). Lanes 5 and 7 are RNA controls to discount genomic DNA contamination.

Description of *Magnetospirillum bellicus* sp. nov. *Magnetospirillum bellicus* (bel'li.cus. L. adj. *bellicus*, martial, warlike, referring to the propensity of the organism to degrade perchlorate, a principal component of munitions and solid rocket fuel).

Spirillum-shaped cells, 0.5 by 3 μm , non-spore forming, non-fermentative, facultatively anaerobic. Cells are motile and may occur singly or in chains. Strictly respiring, complete oxidizer that oxidizes acetate with O_2 , ClO_4^- , ClO_3^- , NO_3^- , or N_2O as the electron acceptor. Perchlorate and chlorate are completely reduced to Cl^- with transient chlorate accumulation during growth on perchlorate. Cells can use substrates such as carboxylic acids—including acetate and propionate—and ethanol for chemoorganotrophic growth, as well as hydrogen for lithotrophic growth (Table 3 shows a complete list). Cells contain the RuBisCo *cbbM* gene. Optimum growth occurs at 42°C, pH 6.8, in freshwater basal medium with 0% NaCl. The G+C content of the genomic DNA of the type strain is 64.8 mol% (determined by HPLC). The type strain is VDY^T (DSM 21662, ATCC BAA-1730) and was isolated from the cathodic chamber of an active perchlorate-reducing BER enrichment.

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