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Isolation and characterization of autotrophic, hydrogen-utilizing, perchlorate-reducing bacteria

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Abstract Recent studies have shown that perchlorate (ClO_4^-) can be degraded by some pure-culture and mixed-culture bacteria with the addition of hydrogen. This paper describes the isolation of two hydrogen-utilizing perchlorate-degrading bacteria capable of using inorganic carbon for growth. These autotrophic bacteria are within the genus *Dechloromonas* and are the first *Dechloromonas* species that are microaerophilic and incapable of growth at atmospheric oxygen concentrations. *Dechloromonas* sp. JDS5 and *Dechloromonas* sp. JDS6 are the first perchlorate-degrading autotrophs isolated from a perchlorate-contaminated site. Measured hydrogen thresholds were higher than for other environmentally significant, hydrogen-utilizing, anaerobic bacteria (e.g., halorespirers). The chlorite dismutase activity of these bacteria was greater for autotrophically grown cells than for cells grown heterotrophically on lactate. These bacteria used fumarate as an alternate electron acceptor, which is the first report of growth on an organic electron acceptor by perchlorate-reducing bacteria.

Introduction

Perchlorate contamination of groundwater is an increasing environmental concern because perchlorate affects the thyroid gland to prevent uptake of iodine (Urbansky and

Schock 1999; Brechner et al. 2000; Logan 2001). Improper storage and disposal of rocket propellants used by the military and industry has led to contamination in 44 states and is estimated to affect 15 million people in the United States (Logan 2001). Characterization of perchlorate-degrading bacteria and their activity has increased dramatically in recent years, yet their ecological history is not well understood. It is known that perchlorate-degrading bacteria utilize two key enzymes: (1) perchlorate reductase to reduce perchlorate to chlorate and reduce chlorate to chlorite (Kengen et al. 1999) and (2) chlorite dismutase to transform chlorite to chloride and molecular oxygen (Rikken et al. 1996; Coates et al. 1999; Stenklo et al. 2001; Hagedoorn et al. 2002). Recent studies using flow-through biological reactors have shown that addition of hydrogen as an electron donor can sustain perchlorate reduction (Giblin et al. 2000; Miller and Logan 2000; Nerenberg and Rittmann 2002). While pure cultures of *Dechloromonas* sp. JM and *Wolinella succinogenes* have shown perchlorate-degrading activity in the presence of added hydrogen, previously only *Dechloromonas* sp. HZ and *Dechloromonas* sp. PC1 have shown autotrophic activity (Wallace et al. 1996; Miller and Logan 2000; Zhang et al. 2002; Nerenberg, personal communication). To our knowledge, no autotrophic bacteria have previously been isolated from a perchlorate-contaminated site. This paper describes two autotrophic, hydrogen-utilizing, perchlorate degraders obtained from a perchlorate-contaminated site, which are also the first perchlorate-degrading bacteria reported to utilize an organic electron acceptor (fumarate).

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Materials and methods

Isolation of perchlorate-reducing bacteria

Bacteria were isolated from a hydrogen-fed microcosm containing groundwater and soil collected from the Longhorn Army Ammunition Plant, a perchlorate-contaminated site (Smith et al. 2001). Standard anaerobic techniques

were used for bacterial isolation where aliquots of the microcosm (0.1 ml) were added to 10 ml "Freshwater" medium (pH 7.0; Bruce et al. 1999) containing 5 mM sodium perchlorate and 40% H₂ headspace (0.41 mmol H₂ to 10 ml liquid; with the remainder as N₂/CO₂, 48%/12%) in 25-ml glass anaerobic pressure tubes (Hungate 1969) incubated at 30°C in the dark. After two transfers, isolated colonies were picked from 1.2% Noble agar anaerobic roll tubes containing 5 mM perchlorate and 40% H₂ (headspace).

Growth of isolates

To measure the growth of isolates on hydrogen and perchlorate, 160-ml serum bottles containing 100 ml Freshwater medium with 10 mM perchlorate and a headspace of H₂, N₂, CO₂ (40%/50%/10%, by vol.) gas were inoculated with 1 ml cells estimated to be in the log phase of growth. Due to concerns about maintaining the integrity of the headspace (and available H₂), each serum bottle in a series was treated as one sample and was sacrificed at a given time to determine the hydrogen, perchlorate, and dry cell weight as volatile suspended solids (VSS) for each recorded data point. These bottles and those for experiments described below were incubated at 30°C with continuous shaking at 160 rpm.

The ability of isolates to utilize lactate, acetate, hydrogen, succinate, propionate, butyrate, formate, casamino acids, glucose, ethanol, benzoate, benzene, or ferrous iron as electron donors for growth (i.e., to yield cells >10× original inocula) on 5 mM perchlorate was investigated. Additionally, the ability of these strains to grow using lactate, acetate, hydrogen, glucose, ethanol, or benzoate with 5 mM nitrate was investigated. Similarly, both isolates were investigated for the potential to grow with 10 mM acetate, using the electron acceptors chlorate, nitrate, oxygen, fumarate, sulfate, sulfite, selenate, and ferric iron. Isolates were also investigated for growth using a headspace of 40% H₂, 20% O₂, 35% N₂, and 5% CO₂.

The ability to grow microaerophilically was investigated by inoculating bacteria in Freshwater medium containing 0.25% Noble agar prepared under N₂/CO₂ gas (80%/20%, v/v) in 25-ml anaerobic pressure tubes with 10 mM lactate and 5 mM perchlorate prior to agar solidification. When this "sloppy agar" was mostly solidified, the tubes were uncapped and exposed to the atmosphere under sterile conditions, recapped, and incubated at 30°C. Strains were assessed for catalase activity using 3% hydrogen peroxide solution (Benson 1998).

¹⁴C experiments

H¹⁴CO₃⁻ (1 μCi, Sigma Chemical) as sodium bicarbonate solution was added to 750 ml of Freshwater medium containing 10 mM perchlorate and 40% H₂ (headspace) in 1-l Pyrex bottles fitted with caps utilizing butyl-rubber stoppered ports to allow syringe sampling and autoclaved

prior to inoculation. Bottles were inoculated with 5 ml cells estimated to be in the log phase of growth. Aqueous samples were collected using a sterile syringe and needle and were filtered using a 0.2-μm syringe filter. Sub-samples of 100 μl were dissolved in 10 ml Scintiverse scintillation cocktail (Fischer Scientific) for quantification of bulk radioactivity by a model LS 6000IC liquid scintillation system (Beckman, Fullerton, Calif.). An additional sub-sample was analyzed for perchlorate as described in the section "Analyses." Gaseous samples of 200 μl (collected using a 250-μl pressure-lock gas syringe) were slowly passed through 10 ml Harvey scintillation cocktail (RJ Harvey, Hillsdale, N.J.) for counting. At the end of the experiment, the contents (ca. 750 ml) of the Pyrex bottles were sacrificed and filtered through GF/C 1.2-μm pore glass filters (Whatman, Clifton, N.J.) for combustion in a model OX-600 biological oxidizer (RJ Harvey) at 900°C, using Harvey cocktail as the ¹⁴CO₂ trapping solution and liquid scintillation fluid.

Hydrogen threshold determination

The hydrogen threshold was estimated as described by Löffler et al. (1999), where strains were grown as described above with 40% hydrogen headspace. Hydrogen was depleted at the end of 1 week and the tubes were spiked with 10% hydrogen. Measurements for hydrogen were taken once daily for 2 weeks until the hydrogen concentration stabilized. Hydrogen concentrations (nanomoles per liter liquid) were calculated as described by Conrad (1996) and Löffler et al. (1999): dissolved H₂ = LP/RT , where dissolved hydrogen is expressed as moles per liter liquid, L is the Ostwald coefficient (0.01895 at 30°C; Wilhelm et al. 1977), P is the hydrogen partial pressure, R is the universal gas constant (0.0821 l atm K⁻¹ mol⁻¹; 1 atm = 101 kPa), and T is the temperature (K).

Chlorite dismutase assays

Wet, unwashed cell suspensions were examined for their ability to degrade chlorite. Chlorite-utilizing activity was determined performing a microassay, as outlined by Chaudhuri et al. (2002).

Analyses

Analysis for perchlorate, chlorate, chlorite, and chloride was performed using a DX-500 ion chromatograph (Dionex, Sunnyvale, Calif.) and an AS11 column (Dionex) with a sodium hydroxide eluent.

Hydrogen analysis was performed using a Hewlett Packard 5890 series II gas chromatograph equipped with a thermal conductivity detector or a RGA3 trace analytical reduction gas detector (Sparks, Md.). The detection limit of hydrogen was approximately 1 ppm. Dry cell mass was determined by measuring the VSS by standard wet chem-

istry techniques (APHA et al. 1989). Absorbance measurements were made using a Genesys 5 spectrophotometer (Spectronic, Rochester, N.Y.).

Microscopic investigation

Wet cell mounts and Gram stains of all isolates were investigated optically using a 20× oil immersion or 40× oil immersion objective with transmitted light using an Axio-Tech microscope (Zeiss, Göttingen, Germany) and Axio-vision software (Zeiss) installed on a personal computer.

16S rRNA gene sequencing and analysis

Cells from 10-ml cultures of perchlorate-reducing bacteria were harvested by centrifugation, resuspended in 1 ml sterile water, and lysed by the addition of 20 µl chloroform and incubation for 10 min at 90°C. Primers specific to bacterial DNA of 16S rRNA were used to amplify DNA by PCR: primer 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and primer 1525R ([5'-AGGAGGTGATCCAGCC-3'; Coates et al. 1999). DNA was extracted using a QIAquick extraction kit (Qiagen, Valencia, Calif.) and submitted for sequencing by a Sanger-based method at the University of Iowa DNA Facility. Partial 16S gene sequence was determined by overlapping bases recovered from sequencing performed using primer 8F and primer 907R (5'-CCGTCA AATCMTTGAGTTT-3'; Schäfer and Muyzer 2001). A partial phylogenetic tree was constructed using a recovered sequence of DNA encoding 16S rRNA from JDS5 and JDS6 along with 16S rRNA gene sequences of perchlorate-degrading and other bacteria deposited in GenBank. A multiple sequence global alignment was performed using ClustalW (Jeanmougin et al. 1998), trimmed using BioEdit (<http://www.jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html>) to retain a commonly shared region of 793 bases across all 31 species, and analyzed for maximum parsimony using PAUP* ver. 4.0 (Swofford 2002).

GenBank sequences from the following organisms were used in the phylogenetic tree (Achenbach et al. 2001; Logan et al. 2001; Zhang et al. 2002): *W. succinogenes* ATCC 29543 (M26636), *Treponema pallidum* (M88726), *Magnetospirillum gryphiswaldense* (Y10109), *Dechlorosporillum* sp. WD (AF170352), *Azospirillum brasiliense*

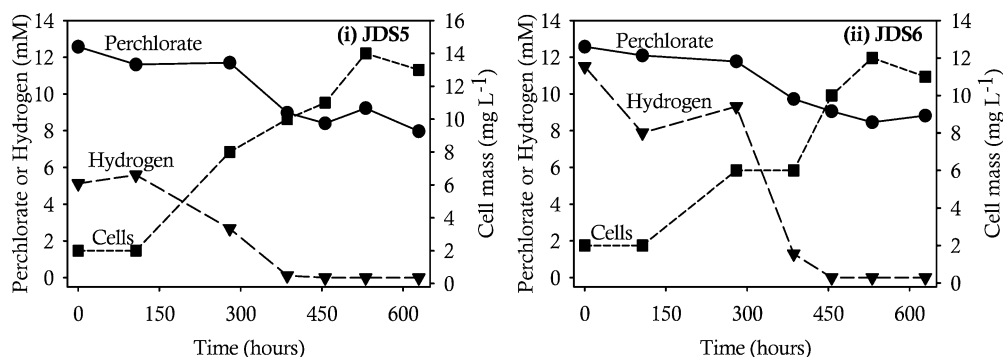
(Z29617), *Comamonas testosteroni* (M11224), *Ideonella dechloratans* (X72724), *Dechloromonas* sp. CL (AF170354), *D. agitata* strain CKB (AF047462), *Dechloromonas* sp. NM (AF170355), *Dechloromonas* sp. JJ (AY032611), *Dechloromonas* sp. JM (AF323489), *Dechloromonas* sp. HZ (AF479766), *Dechloromonas* sp. PC1 (AY126452), *Dechloromonas* sp. MissR (AF170357), *Dechloromonas* sp. SIUL (AF170356), *Ferribacterium limneticum* strain CDA (Y17060), *Rhodocycilus tenuis* strain DSM109 (D16208), *R. purpureus* strain DSM168 (M34132), *Azoarcus evansii* (X77679), *A. denitrificans* strain Td-17 (L33689), *Thauera selenatis* (X68491), *Dechlorosoma suillum* strain PS (AF170348), *Dechlorosoma* sp. PDX (AF323490), *Dechlorosoma* sp. KJ (AF323491), the gill symbiont *Thyasira flexuosa* (L01575), *Dechloromarinus chlorophilus* strain NSS (AF170359), *Pseudomonas stutzeri* (U26415), and *Helicobacter pylori* (M88157). The sequences determined for strains JDS5 and JDS6 were deposited in the GenBank database with accession numbers AY084086 and AY084087.

Results

Isolation of bacteria

Two hydrogen-utilizing perchlorate-degrading bacteria were isolated from microcosms containing soil and groundwater from a perchlorate-contaminated site (Longhorn Army Ammunition Plant, Karnack, TX). Strains JDS5 and JDS6 were isolated by their ability to repeatedly use inorganic carbon (bicarbonate) as a carbon source with hydrogen as the sole electron donor and perchlorate as the electron acceptor. Hydrogen and perchlorate utilization over time was observed with an increase in cell mass (Fig. 1). Both isolates were Gram-negative rods, approx. 1 µm in length, that displayed motility in wet cell mounts. The minimum doubling times for JDS5 and JDS6 with hydrogen and perchlorate were 10 h and 17 h, respectively. Both strains also grew heterotrophically and the doubling times of JDS5 and JDS6 were 4.5 h and 4.8 h, respectively, when grown on perchlorate and lactate. No chlorate or chlorite was detected at any time and analyses for chloride indicated that 92±21% of added perchlorate was reduced to chloride.

Fig. 1 Growth of *Dechloromonas* sp. JDS5 (left) and *Dechloromonas* sp. JDS6 (right) on hydrogen and perchlorate under autotrophic conditions. L Liter



Confirmation of autotrophic growth

Evidence for autotrophic growth was obtained using an electron balance (Table 1). Hydrogen consumption was balanced with perchlorate reduction and cell growth, based upon stoichiometry of electron usage (Sawyer et al. 2003). Approximately 87% of the H₂ consumption by JDS5 was accounted for by the perchlorate reduced and cells produced; and, with JDS6, approximately 94% of the electrons were accounted for (n.b., if heterotrophic growth were significant, recoveries would exceed 100%).

Under all growth conditions, but particularly when grown on hydrogen, isolates JDS5 and JDS6 tended to grow in flocs or clumps; and for this reason VSS was utilized as optical density measurements at 600 nm could not be used to quantify cell mass. JDS5 and JDS6 also tended to form films along the side and bottom of the growth tube or vessel and adherence persisted even after vigorous shaking. Thus, it is likely that the measured dry cell weight (VSS) may have been less than the actual cell weight for some measurements.

Autotrophic growth of JDS5 and JDS6 was confirmed by recovery of ¹⁴C from cells (i.e., particulate fraction) grown on H₂ and perchlorate, using H¹⁴CO₃⁻ as a fraction of inorganic carbon in the medium (data not shown). Total recovery of ¹⁴C from the gas, aqueous, and particulate phases was 102% and 101% for JDS5 and JDS6, respectively, of the initial measured ¹⁴C. Negative controls without CO₂/HCO₃⁻ containing 0.1 M phosphate buffer (pH 7.2) were unable to grow on H₂ and perchlorate.

Electron donor and acceptor utilization

To allow comparison of these isolates with previously identified perchlorate-degrading bacteria, we checked the isolates for their ability to use a variety of substrates. JDS5 and JDS6 utilized lactate (10 mM), acetate (10 mM), hydrogen (40.4 kPa), propionate (5 mM), and butyrate (5 mM) as electron donors when using perchlorate (5 mM) as an electron acceptor. "Poor" growth (characterized by very

Table 1 Electron mass balance^a for strains JDS5 and JDS6 grown on 40% hydrogen headspace and 10 mM perchlorate under autotrophic conditions at 30°C

Strain	Hydrogen consumed	Perchlorate reduced	Cell mass produced	Mass balance of electrons (%)
JDS5	38.2±2.3	29.6±0.3	6.34±2.89	94±12
JDS6	40.2±1.2	30.0±1.1	3.83±0.26	87±4

^aUnits are milliequivalents of electrons (meeq) l⁻¹. All values are the average difference between measurements at 0 h and 142 h for three replicates ±1SD. Based upon Sawyer et al. (2003): 1 mol H₂ = 2 mol electrons and 1 mol cells (as C₅H₇O₂N) = 20 mol electrons = 113 g cells, assuming mineralization of perchlorate (ClO₄⁻ + 8e⁻ + 8H⁺ → Cl⁻ + 4H₂O), 1 mol ClO₄⁻ = 8 mol electrons. Cell mass produced was estimated as recovered dry cell weight from VSS measurements. The electron mass balance was determined as ((perchlorate reduced + cell mass produced)/hydrogen consumed) × 100

little production of cell flocs) was observed with formate (5 mM) and casamino acids (1 g l⁻¹) with 5 mM perchlorate. No growth was observed with perchlorate (5 mM) and glucose (5 mM), ethanol (5 mM), benzoate (5 mM), benzene (0.1 mM), or Fe(II) (5 mM). Both isolates also grew on nitrate (5 mM) with lactate (5 mM), acetate (5 mM), and hydrogen (40.4 kPa), but not with glucose (5 mM), ethanol (5 mM), or benzoate (5 mM). The isolates were unable to ferment lactate or acetate.

JDS5 and JDS6 were also examined for their ability to utilize alternate electron acceptors. Growth of JDS5 and JDS6 on acetate (10 mM) was observed with chlorate (5 mM), nitrate (5 mM), and fumarate (5 mM) in addition to perchlorate. This is the first report of a perchlorate-reducing bacteria capable of using an organic electron acceptor (fumarate). However, this behavior has not been investigated for all known isolates (Rikken et al. 1996; Bruce et al. 1999; Coates et al. 1999; Logan et al. 2001; Wolterink et al. 2002; Zhang et al. 2002). Previous research has shown growth on fumarate as an electron donor when coupled with perchlorate or chlorate reduction (Bruce et al. 1999; Coates et al. 1999). No growth was observed with acetate (10 mM) and sulfate (5 mM), sulfite (5 mM), selenate (5 mM), or Fe(III) (5 mM). Additionally, JDS5 and JDS6 were unable to grow aerobically (or in a closed headspace with 20% oxygen) on 10 mM acetate, 10 mM lactate or 40% hydrogen. This is significant, as all previously isolated *Dechloromonas* species are capable of growing aerobically on atmospheric levels of oxygen (Bruce et al. 1999; Coates et al. 1999; Logan et al. 2001; Zhang et al. 2002).

While unable to grow at atmospheric concentrations, oxygen tolerance of these strains was observed. Both strains showed catalase activity when a 3% hydrogen peroxide solution was added to wet cell suspensions as a test of aero-tolerance. The formation of bubbles was slow for JDS5 and JDS6 compared with immediate and rapid bubble formation by *D. agitata* (Achenbach et al. 2001). JDS5 and JDS6 grew microaerophilically within an anaerobic/aerobic gradient created with sloppy agar prepared under anaerobic conditions exposed to an aerobic headspace at approximately 0.5 cm below the gas and agar interface (data not shown). The only other known microaerophilic perchlorate-degrading bacterium is *W. succinogenes* (Wallace et al. 1996).

Differences between heterotrophic and autotrophic growth

The strains produced fewer cells when grown with hydrogen as compared to lactate, and JDS5 always produced more biomass than JDS6 (Table 2). While differences between autotrophic and heterotrophic growth would be expected based upon thermodynamic considerations, the mass of new cells produced may be relevant for the design of perchlorate remediation systems (e.g., activated-sludge system) that requires routine wasting of bacteria. Since both JDS5 and JDS6 degraded more perchlorate per cell

Table 2 Biomass production and chlorite dismutase activity by *Dechloromonas* sp. JDS5, *Dechloromonas* sp. JDS6, and *D. agitata* CKB grown with different electron donors. NA Not applicable, *D. agitata* CKB was grown only on lactate

Electron donor	Biomass production (mg VSS mmol ⁻¹ ClO ₄ ⁻)			Chlorite dismutase activity (mmol ClO ₂ ⁻ mg ⁻¹ VSS s ⁻¹)		
	JDS5	JDS6	CKB	JDS5	JDS6	CKB
Lactate (10 mM) ^a	14	3.3	31	0.54	0.51	4.7
Hydrogen (40.4 kPa) ^b	1.7	1.0	NA	5.1	1.7	NA

^aCells grown with 5 mM perchlorate

^bCells grown with 10 mM perchlorate

using hydrogen than with lactate, hydrogen would be the preferred electron donor when cell production must be minimized.

The chlorite (ClO₂⁻) dismutase activity of perchlorate- and chlorate-degrading organisms is critical to survival of these bacteria and is likely unique to these organisms (Rikken et al. 1996; Coates et al. 1999; Stenklo et al. 2001; Hagedoorn et al. 2002). JDS5 and JDS6 were examined for their ability to directly utilize ClO₂⁻. Both isolates showed an ability to transform ClO₂⁻ when grown autotrophically or heterotrophically on perchlorate. Interestingly, both JDS5 and JDS6 showed greater ClO₂⁻ transformation when grown on hydrogen than when grown on lactate. The maximum chlorite transformation rate (initial concentration = 10 mM) was approximately ten-fold greater for cells grown autotrophically on hydrogen, compared with cells grown heterotrophically on lactate (Table 2). The maximum chlorite utilization rate by *D. agitata* CKB (grown on 10 mM lactate, 5 mM perchlorate) was 4.7 mmol ClO₂⁻ mg⁻¹ VSS s⁻¹, which is in the same order as strains JDS5 and JDS6 grown on hydrogen and perchlorate. Production of oxygen during the assays was not quantified. However, bubbles were observed to form during the assay, indicating that molecular oxygen was being produced by

the dismutation of ClO₂⁻. Similar observations of gas evolution were reported by Coates et al. (1999).

Hydrogen threshold

The minimum level of hydrogen required to sustain bacterial activity has been reported for several bacteria and different terminal electron acceptor processes (TEAPs), which is relevant to how these bacteria compete for hydrogen in nature and in engineered remediation systems. The observed hydrogen thresholds for JDS5 and JDS6 were 119±6.5 nM and 113±17 nM, respectively. These values were higher than anticipated based upon thermodynamic considerations (Table 3). In general, hydrogen thresholds are lower for more thermodynamically favorable TEAPs (Lovley et al. 1994; Löffler et al. 1999). Hydrogen thresholds for JDS5 and JDS6 did not follow this trend and the data suggest that these perchlorate-reducing autotrophs may not compete well for hydrogen in the environment.

Phylogenetic and phenotypic characterization

The DNA sequence encoding 16S rRNA recovered from the two isolates suggests they are both members of the genus *Dechloromonas* within the β-Proteobacteria (Fig. 2). *Dechloromonas* sp. JDS5 and *Dechloromonas* sp. JDS6 did not show particular similarity within the *Dechloromonas* genus to the two other known autotrophic perchlorate-reducing bacteria, *Dechloromonas* sp. HZ and *Dechloromonas* sp. PC1 (Zhang et al. 2002; Nerenberg, personal communication). All these autotrophic perchlorate-degraders possess similar phenotypic characteristics—both the ability to grow autotrophically (hydrogen) or heterotrophically (acetate) and the ability to use chlorate or nitrate as alternate electron acceptors. The greatest known differences are in the culturing and enumeration of

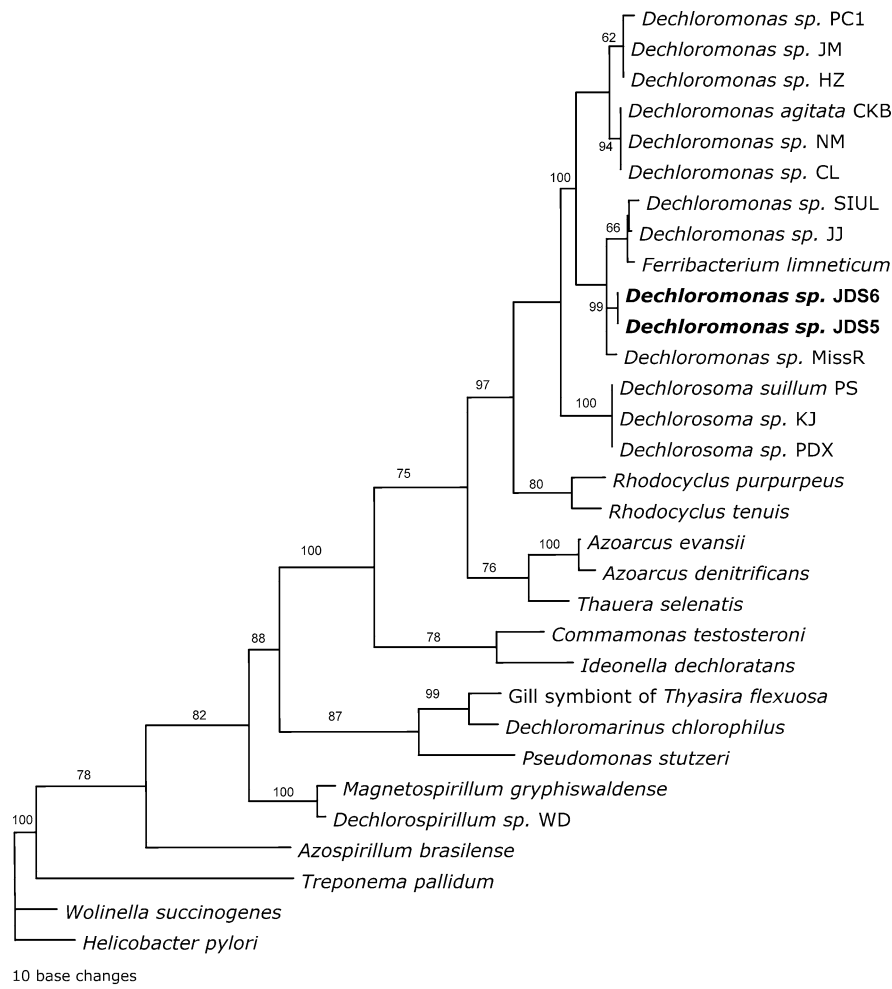
Table 3 Hydrogen threshold concentrations and free energies for hydrogen coupled to different TEAPs after Löffler et al. (1999)

TEAP	H ₂ threshold concentration		ΔG ^{o'} (kJ mol ⁻¹ H ₂)	Reference
	ppm	Dissolved (nM)		
Acetogenesis (CO ₂)	430–4,660	336–3,640	-24.92	Cord-Ruwisch et al. (1988), Breznak (1994)
Methanogenesis (CO ₂)	6–120	5–95	-32.70	Lovley (1985), Cord-Ruwisch et al. (1988), Lovley and Goodwin (1988), Conrad (1996)
Sulfate reduction (SO ₄ ²⁻)	1.3–19	1–15	-38.04	Cord-Ruwisch et al. (1988), Lovley and Goodwin (1988), Conrad (1996)
Dehalogenation (tetrachloroethene)	<0.4	<0.3	-157.71 to -173.56 ^b	Löffler et al. (1999)
Nitrate reduction (NO ₃ ⁻)	<0.06	<0.05	-224.14	Lovley and Goodwin (1988), Lovley et al. (1994)
Fe ³⁺ reduction (Fe ³⁺)	0.13–1	0.1–0.8	-228.28	Lovley and Goodwin (1988), Lovley et al. (1994), Conrad (1996)
Perchlorate reduction (ClO ₄ ⁻)	147–154	113–119	-267.86	This study

^aΔG^{o'} values calculated from free energies given by Sawyer et al. (2003), assuming pH 7.0.

^bΔG^{o'} calculated for range of sequential dehalogenation steps of tetrachloroethene to ethene and chloride.

Fig. 2 Phylogenetic relationships between *Dechloromonas* sp. JDS5, *Dechloromonas* sp. JDS6, and other known Proteobacteria based upon maximum parsimony analysis of aligned regions of DNA from 16S rRNA gene sequences. Bootstrap support values from 100 replicates are indicated at branch nodes



these bacteria. Zhang et al. (2002) reported *Dechloromonas* sp. HZ as being particularly difficult to culture due to its inability to grow on plates. This was not observed with *Dechloromonas* sp. JDS5 and *Dechloromonas* sp. JDS6, which readily attached to surfaces when grown in liquid medium. However, the microaerophilic characteristics of *Dechloromonas* sp. JDS5 and *Dechloromonas* sp. JDS6 precluded culturing of these bacteria by standard aerobic plating techniques. The minimum doubling times of these four bacteria grown autotrophically were of the same order of magnitude: 8.9 h (*Dechloromonas* sp. HZ at 28°C), 10 h (*Dechloromonas* sp. JDS5 at 30°C), 17 h (*Dechloromonas* sp. JDS6 at 30°C), and 24 h (*Dechloromonas* sp. PC1 at 22°C; Zhang et al. 2002; Nerenberg, personal communication).

Discussion

Two isolates capable of reducing perchlorate to chloride when grown autotrophically using hydrogen were obtained from a microcosm started from soil and water collected at a perchlorate-contaminated site. These are first reported autotrophic perchlorate-degrading bacteria isolated from a perchlorate-contaminated site and phylogenetic analysis

places JDS5 and JDS6 in the genus *Dechloromonas*. *Dechloromonas* sp. JDS5 and *Dechloromonas* sp. JDS6 are the first microaerophilic *Dechloromonas* species incapable of respiring atmospheric levels of oxygen (Bruce et al. 1999; Coates et al. 1999; Logan et al. 2001; Zhang et al. 2002). While JDS5 and JDS6 appeared to have insufficient production of oxygen-utilizing enzymes to grow at atmospheric concentrations of oxygen, these strains did display catalase activity and they grew microaerophilically at low oxygen tensions. Previously, perchlorate or chlorate-degraders unable to utilize atmospheric oxygen have only been reported outside the two main perchlorate-degrading genera *Dechloromonas* and *Dechlorosoma* (Wallace et al. 1996; Logan 1998; Wolterink et al. 2002).

In general, JDS5 and JDS6 appear very robust, as they were also able to grow heterotrophically using a variety of electron donors and acceptors. This is also the first report of perchlorate-reducing bacteria capable of using an organic electron acceptor (fumarate).

Differences in chlorite dismutase activity for both JDS5 and JDS6 were observed for cells grown autotrophically compared with those grown heterotrophically, as autotrophically grown cells showed increased chlorite dismutase activity. The specific explanation for this result could not be determined from these experiments. It is possible

that more dismutase is produced during autotrophic growth. There is also the possibility that alternate (or additional) dismutase isozymes are produced by these strains under different growth conditions. For example, the ability of bacteria to produce alternate enzymes under differing growth conditions has been shown for the nitrogenase enzymes of nitrogen-fixing bacteria, the superoxide dismutases of aero-tolerant bacteria, the oxidoreductases of *Azotobacter vinelandii*, and 4-hydroxybenzoate degradation by *Acinetobacter* or *Alcaligenes* species (Harwood and Parales 1996; Bertsova et al. 1998; Kim et al. 1999; White 2000; Leclere et al. 2001).

The recent isolation of these and other bacteria capable of autotrophic growth on hydrogen and perchlorate expands the known activities of perchlorate-degrading bacteria. Recent reports indicate that perchlorate-degrading bacteria are found in a wide variety of environments (Coates et al. 1999). However, the ubiquity of the isolates (or similar phenotypes) reported here has not been investigated. Additionally, the comparatively high hydrogen threshold of these isolates suggests these bacteria may not compete well for environmental levels of hydrogen. Any potential benefits of increased chlorite dismutase activity with the addition of hydrogen compared with lactate are unlikely to be observed if hydrogen is not available to these bacteria—because active metabolism of perchlorate appears to be required for the production of chlorite dismutase (Chaudhuri et al. 2002). The isolation of these organisms does support the hypothesis that, while perchlorate-degrading bacteria appear ubiquitous in nature, many bacteria capable of perchlorate degradation display alternate activities that remain to be discovered.

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