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Novel chemolithotrophic, thermophilic, anaerobic bacteria *Thermolithobacter ferrireducens* gen. nov., sp. nov. and *Thermolithobacter carboxydivorans* sp. nov.

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Abstract Three thermophilic strains of chemolithoautotrophic Fe(III)-reducers were isolated from mixed sediment and water samples (JW/KA-1 and JW/KA-2^T; Calcite Spring, Yellowstone N.P., WY, USA; JW/JH-Fiji-2: Savusavu, Vanu Levu, Fiji). All were Gram stain positive rods (~0.5 × 1.8 μm). Cells occurred singly or in V-shaped pairs, and they formed long chains in complex media. All utilized H₂ to reduce amorphous iron (III) oxide/hydroxide to magnetite at temperatures from 50 to 75°C (opt. ~73°C). Growth occurred within the pH^{60°C} range of 6.5–8.5 (opt. pH^{60°C} 7.1–7.3). Magnetite production by resting cells occurred at pH^{60°C} 5.5–10.3 (opt. 7.3). The iron (III) reduction rate was 1.3 μmol Fe(II) produced × h⁻¹ × ml⁻¹ in a culture with 3 × 10⁷ cells, one of the highest rates reported. In the presence or absence of H₂, JW/KA-2^T did not utilize CO. The G + C content of the genomic DNA of the type strain is 52.7 ± 0.3 mol%. Strains JW/KA-1 and JW/KA-2^T

each contain two different 16S rRNA gene sequences. The 16S rRNA gene sequences from JW/KA-1, JW/KA-2^T, or JW/JH-Fiji-2 possessed >99% similarity to each other but also 99% similarity to the 16S rRNA gene sequence from the anaerobic, thermophilic, hydrogenogenic CO-oxidizing bacterium '*Carboxydothemus restrictus*' R1. DNA–DNA hybridization between strain JW/KA-2^T and strain R1^T yielded 35% similarity. Physiological characteristics and the 16S rRNA gene sequence analysis indicated that the strains represent two novel species and are placed into the novel genus *Thermolithobacter* within the phylum '*Firmicutes*'. In addition, the levels of 16S rRNA gene sequence similarity between the lineage containing the *Thermolithobacter* and well-established members of the three existing classes of the '*Firmicutes*' is less than 85%. Therefore, *Thermolithobacter* is proposed to constitute the first genus within a novel class of the '*Firmicutes*', *Thermolithobacteria*. The Fe(III)-reducing *Thermolithobacter ferrireducens* gen. nov., sp. nov. is designated as the type species with strain JW/KA-2^T (ATCC 700985^T, DSM 13639^T) as its type strain. Strain R1^T is the type strain for the hydrogenogenic, CO-oxidizing *Thermolithobacter carboxydivorans* sp. nov. (DSM 7242^T, VKM 2359^T).

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Introduction

Anaerobic, microbially-mediated processes, such as carbon monoxide oxidation and autotrophic iron (III) reduction, may have been important on primitive Earth, which contained a reduced atmosphere and very low levels of oxygen (Lovley 1991; Gold 1992). Increasingly

biotic dissimilatory iron reduction has become a topic of interest due to its possible role in biogeochemical cycling and potential importance in the evolution of microbial life (Lovley 1991).

Iron is the third most abundant element in the Earth's crust (McGeary and Plummer 1997), and it is generally believed that iron-reducing bacteria are more abundant in nature than their representation in culture collections. Of particular interest are thermophilic, iron-reducing, chemolithoautotrophs, which could have been involved in the precipitation of ancient Banded Iron Formations. Bacteria from a variety of phylogenetic groups are capable of iron reduction (Boone et al. 1995; Lonergan et al. 1996; Slobodkin et al. 1997, 1999). Three out of eight published thermophilic, iron-reducing bacteria (Table 1) belong to the Gram-type positive 'Firmicutes' (Gibbons and Murray 1978; Garrity et al. 2002). Others belong to the Gram-type negative *Flexistipes*, *Deferribacter thermophilus* (Greene et al. 1997); *Proteobacteria*, '*Geothermobacterium ferrireducens*' (Kashefi et al. 2002b) and *Geothermobacter ehrlichii* (Kashefi et al. 2003); *Thermatogales*, *Thermotoga maritima* (Huber et al. 1986; Vargas et al. 1998); and the *Deinococcus-Thermus* clade, *Thermus scotoductus* (Balkwill et al. 2004). In addition, two archaeal, thermophilic iron-reducers are known: *Geoglobus ahangari* (Kashefi et al. 2002a), and *Pyrobaculum islandicum* (Kashefi and Lovley 2000).

Autotrophic, dissimilatory iron reduction was first demonstrated for *Thermoterrabacterium ferrireducens* and *Thermoanaerobacter siderophilus* (Slobodkin et al. 1997, 1999). Moreover, Slobodkin and Wiegel (1997) showed that several different Fe(III)-reducing microorganisms must exist growing at temperatures up to 90°C. Since that time, the autotrophic, dissimilatory iron-reducers *Geoglobus ahangari* (Kashefi et al. 2002a) and '*Geothermobacterium ferrireducens*' (Kashefi et al. 2002b) have been isolated, and *Pyrobaculum islandicum* has been shown to grow autotrophically (Kashefi and Lovley 2000).

Another type of chemolithotrophic growth that often presumably exists in tandem with dissimilatory, chemolithotrophic iron-reduction is hydrogenogenic, CO-oxidation. Members of both groups have been found to inhabit unique microbial communities and both often rely on the energy of reduced inorganic compounds (Slobodkin and Wiegel 1997; Slobodkin et al. 1999). Hydrogenogenic, CO-oxidizing anaerobic prokaryotes base their metabolism on the anaerobic oxidation of carbon monoxide to equimolar hydrogen gas and carbon dioxide production, according to the reaction $\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2$ ($\Delta G^0 = -20$ kJ). The name "hydrogenogens" has been proposed for this physiological group because hydrogen is the only reduced product of their metabolism, and physiological groups of anaerobic prokaryotes are frequently named after the major reduced product of their metabolism (Svetlichnyi et al. 2001). CO-oxidizing hydrogenogenic prokaryotes belong to the bacterial genera *Carboxydo-*

thermus (Svetlichnyi et al. 1991, 1994), *Caldanaerobacter* (Sokolova et al. 2001), *Carboxydocella* (Sokolova et al. 2002), *Thermosinus* (Sokolova et al. 2004a) and *Thermincola* (Sokolova et al. 2005) and to the hyperthermophilic archaeal genus *Thermococcus* (Sokolova et al. 2004b). Hydrogenogenic CO-oxidizing prokaryotes have been shown to grow obligately dependent on CO (Sokolova et al. 2002), fermentatively (Sokolova et al. 2001, 2004a), and employing the reduction of Fe(III) and various other electron acceptors (Sokolova et al. 2004a, b; Henstra and Stams 2004).

Three strains of thermophilic iron-reducers were isolated from hot springs in Yellowstone National Park and Fiji and have been partly described (J. Hanel, 2000, thesis, The University of Georgia; Wiegel et al. 2003). Based on their physiological features and 16S rRNA gene sequence analysis they were proposed as '*Ferribacter thermautotrophicus*' gen. nov., sp. nov. (Wiegel et al. 2003). However, their 16S rRNA gene sequences were similar to the sequence of the hydrogenogenic CO-oxidizing strain R1^T, which was described previously as '*Carboxydothermus restrictus*' sp. nov. (Svetlichnyi et al. 1994). Currently neither name has been validated. The similarity of the above-mentioned strains 16S rRNA gene sequences, and their lack of similarity with other described 'Firmicutes' indicate that they should be (1) assigned as two novel species within a novel genus and (2) proposed as a novel lineage (class) within the 'Firmicutes'. Because the previously suggested names would exclude the specific properties of the other species, we propose to assign them to the novel genus *Thermolithobacter*, a name which includes the key properties of both species.

Materials and methods

Environmental samples

A combined sample of water, organic filamentous material, and sediment containing 10–15 ppm Fe from a runoff of a hot spring close to the Yellowstone river at the Calcite Spring area from Yellowstone National Park (44°54.291' N, 110°24.242' W) contained white and black bacterial filaments. The thermal spring sampling point had a temperature gradient from 60 to 85°C with a pH of 7.6. The sample from Fiji contained water and sediment (Na/K ratio was 25.7, 1,158 ppm Na, 1,606 ppm Ca, 5,250 ppm Cl, 228 ppm SO₄, and 0.06 ppm Fe) (Cox 1981; B.K.C. Patel, 1985, thesis, Waikato University, Hamilton, New Zealand) from Nakama springs (main spring; used by local residents for cooking) south of the soccer field in Savusavu on Vanu Levu. The estimated temperature for its underground origin is around 170°C with a pH ~7.5. Samples were collected in sterile, N₂-flushed 100 ml Pyrex jars, capped with butyl rubber stoppers and brought to the laboratory in Athens, GA, where they were stored at 4–7°C for several weeks before the enrichments were started.

Table 1 Differentiation of JW/KA-2^T from other Fe(III)-reducing thermophilic microorganisms

Properties	Strain JW/KA-2 ^T	<i>Bacillus infernus</i> Boone et al. (1995)	<i>Thermoterra bacterium ferrireducens</i> Slobodkin et al. (1997)	<i>Deferribacter thermophilus</i> Greene et al. (1997)	<i>Thermoanaerobacter siderophilus</i> Slobodkin et al. (1999)	<i>Thermotoga maritima</i> Huber et al. (1986)	' <i>Geothermobacterium ferrireducens</i> ' Kashfehi et al. (2002b)	<i>Geothermobacter ehrlichii</i> Kashfehi et al. (2003)	<i>Thermus scotoductus</i> Balkwill et al. (2004)
Chemolithoautotrophic	+	-	+	-	+	-	+	-	-
Organic electron donors	None	Sugars	Sugars	Sugars	Sugars	Sugars	None	Sugars, amino acids, peptides	Sugars and amino acids
Metabolism	Obligate anaerobe	Obligate anaerobe	Obligate anaerobe	Microaerophilic	Obligate anaerobe	Obligate anaerobe	Obligate anaerobe	Obligate anaerobe	Facultative anaerobe
Spore formation	Not observed	Yes	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed
Flagella	2-3, peritrichous	None	None	Monotrichous (polar)	2-3, peritrichous	Monotrichous (subpolar)	Monotrichous	Monotrichous (subpolar)	None
Motility	Tumbling	None	Tumbling	None	Tumbling	+	+	+	None
Cell size (avg)	0.5 × 1.8 μm	0.7 × 6.0 μm	0.3 × 2.0 μm	0.4 × 3.0 μm	0.5 × 6.5 μm	0.6 × 5.0 μm	0.5 × 1.0 μm	0.5 × 1.2 μm	0.5 × 1.5 μm
Temp. range (°C)	50-75, opt. 72	39-65, opt. 61	50-74, opt. 65	50-65, opt. 60	39-78, opt. 70	55-90, opt. 80	65-100, opt. 85-90	35-65, opt. 55	42-73, opt. 65
pH range	6.5-8.5, opt. 7.3	7.3-7.8, opt. 7.3	5.5-7.6, opt. 6.1	5.0-8.0, opt. 6.5	4.8-8.2, opt. 6.4	5.5-9.0, opt. 6.5	ND (grew 6.8-7.0)	5.0-7.75, opt. 6.0	6-8, opt. 7.5
Gram stain	+	+	+	-	+	-	-	-	-
Reduction of Mn(IV)	-	+	-	+	+	ND	ND	-	ND
G + C content (mol%)	53	ND	41	34	32	46	ND	62.6	64.5

Strain R1^T was isolated from a terrestrial hot spring at the Raoul Island, Archipelago Kermadec (New Zealand).

Media and cultivation

Mineral medium was prepared anaerobically by the Hungate technique (Ljungdahl and Wiegel 1986) under an atmosphere of hydrogen and carbon dioxide gases (80:20 v/v), or under 100% CO. It contained (per liter of de-ionized water): 0.33 g KH₂PO₄, 0.33 g NH₄Cl, 0.33 g KCl, 0.33 g MgCl₂·2H₂O, 0.33 g CaCl₂·2H₂O, 2.0 g NaHCO₃, 1 ml vitamin solution (Wolin et al. 1963), and 1.2 ml trace element solution (Slobodkin et al. 1997). The pH^{25°C} was adjusted to 7.0 with 10% (w/v) NaOH under an atmosphere of either hydrogen and carbon dioxide gases (80:20 v/v) or 100% CO. To this mineral medium, 90 mM amorphous Fe(III) oxide/hydroxide (mineral Fe medium; prepared as described by Slobodkin et al. 1997) or 20 mM 9,10-anthraquinone 2,6-disulfonic acid (AQDS) were typically added as electron acceptors. Medium was routinely sterilized by autoclaving at 121°C for 1 h due to the possible presence of heat stable spores (Byrer et al. 2000).

JW/KA-1, JW/KA-2^T, and JW/JH-Fiji-2 were isolated from mineral Fe-medium enrichments positive for Fe reduction. Briefly, positive enrichments were used to inoculate (2% v/v) mineral AQDS medium. Agar shake roll tubes using AQDS medium supplemented with 2.5% Bacto agar were inoculated and incubated at 60°C. After 24 h, isolated colonies were picked in an anaerobic chamber (Coy Products) under a N₂/H₂ atmosphere (95:5 v/v) and used to inoculate AQDS medium. Cultures were incubated for 48 h at 60°C. Those tubes displaying AQDS reduction and cell growth were used to inoculate (2% v/v) mineral Fe medium. After 48 h, tubes displaying black magnetic precipitation and cell growth were repeatedly used for subsequent (nine times for JW/KA-2^T) picking of colonies from agar shake-roll-tube-dilution rows to ensure obtaining axenic cultures. The picked colonies were re-suspended in 0.5 ml of pre-reduced media and used to inoculate the subsequent round of agar shake-roll-tube-dilution rows. The cell suspensions were examined microscopically to ensure that they contained mainly single cells and no clumps of cells. Stock cultures were stored in 50% v/v glycerol in AQDS and mineral Fe media at -75°C. For routine use, cultures were maintained in both iron oxyhydroxide and AQDS media.

The enrichments and pure cultures of JW/KA-1, JW/KA-2^T, and JW/JH-Fiji-2 were typically grown in Hungate or Balch tubes at 60°C, and all transfers were carried out with disposable syringes and hypodermic needles. No reducing agents were added because the reducing agents dithionite, cysteine, Ti(III) citrate (Gaspard et al. 1998) and HCl-cysteine reduce Fe(III). Strain R1^T was grown at 70-72°C in 50 ml serum flasks containing 10 ml of the liquid mineral medium described

above, except that AQDS and Fe(III) oxide/hydroxide were omitted (Svetlichnyi et al. 1994). The medium was reduced with 0.5 g/l Na₂S 9 H₂O under 100% of CO.

Determination of growth

Growth was determined by direct cell counts and visually by determinations of reduced iron (black magnetic precipitation). Besides cell counting and measuring the change in OD_{600nm}, the growth of the CO-utilizing strain was additionally measured by following CO utilization and H₂ production employing gas chromatography (Sokolova et al. 2004a).

Microscopy

Light microscopic observations were performed using slides coated by 2% (w/v) ultra pure agar and an Olympus VANOX equipped with phase-contrast objectives. Electron microscopy of negatively stained cells was performed with a JEOL CX-100 transmission electron microscope (Valentine et al. 1968). Light and electron microscopy of strain R1^T were performed as described by Svetlichnyi et al. (1994).

Quantification of Fe(II)

Fe(II) was determined employing the 2,2-dipyridyl iron assay of Balashova and Zavarzin (1980). In this assay, all species of iron are dissolved in 0.5 ml of 0.6 M HCl, but only Fe(II) reacts with the added 2,2-dipyridyl. Iron was quantified using an Fe(II) (FeCl₂) standard curve.

Temperature and pH ranges for growth

The temperature range for the iron-reducers was determined in both mineral Fe- and AQDS-media at pH^{25°C} 7.3 using a temperature gradient incubator (Scientific Industries, Inc., Bohemia, NY) with shaking (~20 strokes/min). To compensate for the effect of temperature on pH measurements, pH^{60°C} was determined with a pH meter calibrated with standards and the electrode equilibrated at 60°C (Wiegel 1998). Sterile anaerobic 1 M NaOH and 3 M HCl were used to adjust the pH of the medium prior to inoculation. The temperature and pH^{25°C} ranges for the growth of strain R1^T were determined as described by Svetlichnyi et al. (1994) without shaking.

Carbon sources (substrate utilization)

Growth on potential carbon sources was assessed using Hungate tubes containing mineral medium and Fe(III) in which the autotrophic carbon source, CO₂, was omitted and various other substrates were supplied. In

the case of strain R1^T potential carbon sources which could be utilized during growth on H₂ were assessed using 50 ml serum flasks containing 10 ml of the same mineral medium, except 20 mM AQDS was supplied instead of Fe(III). Cell numbers and iron or AQDS reduction were used to determine growth. Negative controls contained identical media and carbon supplements but were inoculated with autoclaved suspensions of cells. This control tested the possibility of abiotic Fe(III) or AQDS reduction, which is common with carbohydrates at high temperatures (Slobodkin et al. 1997). To avoid false positive results, cultures were transferred into the same media using 1% (v/v) inocula before being scored as positive.

Electron acceptors

The ability to utilize various electron acceptors was tested in mineral medium without the addition of Fe(III). Utilization was assumed when cell counts increased from 1.0 × 10⁶ to 2.0 × 10⁷ in the second and third subsequent subcultures, and when the reduction of ferric citrate and AQDS was accompanied by changes in color from black to clear (reduced form) and yellow to brown or black (reduced form), respectively. When nitrate, thiosulfate, sulfate or elemental sulfur was added as the potential electron acceptor, the medium was reduced with sodium sulfide. The inoculum was 1% (v/v). A culture without addition of an electron acceptor was used as a control.

Lipid extraction and fractionation

Duplicate samples of strain JW/JH-Fiji-2 (0.5 g/ml) were extracted using a modified Bligh and Dyer (1959) extraction method (White et al. 2005). Lipids were separated on a silicic acid column with chloroform, acetone, and methanol into neutral lipid, glycolipid, and polar lipid fractions (Guckert et al. 1985). Solvents were removed under a gentle stream of nitrogen, and the fractions were stored at -20°C. The neutral lipid fraction was resolved in methanol and analyzed for respiratory quinones by HPLC-tandem mass spectrometry (Geyer et al. 2004). The phospholipid fatty acids (PLFAs) in the polar lipid fraction were derivatized with trimethylchlorosilane in methanol to produce fatty acid methyl-esters (FAMES) and analyzed on a GC-MS system after the addition of FAME 19:0 as an internal standard (Geyer et al. 2005).

G + C content

The G + C mol% was determined by the HPLC method of Mesbah et al. (1989). DNA for the analysis was extracted using the mini-BeadBeater DNA extraction system (Biospec Products). DNA from strain R1^T was isolated as described by Marmur (1961).

DNA extraction, sequencing, and 16S rRNA gene sequence phylogenetic analysis

Genomic DNA was extracted from cell pellets obtained from cultures of JW/KA-2^T, JW/KA-1 and JW/JH-Fiji-2 (after multiple rounds of isolating single cell colonies) using the DNeasy Tissue kit (Qiagen). Purified DNA was used to amplify the small-subunit rRNA gene by PCR as previously described (Götz et al. 2002). PCR products (approximately 1,500 base pairs) were purified using the PCRpure Spin Kit (Intermountain Scientific, Kaysville, Utah) and cloned into pCR2.1 vector using the TOPO-TA cloning kit (Invitrogen). Plasmid DNA was prepared using an alkaline-lysis method (Sambrook et al. 1989). Sequencing reactions were performed using the ABI PRISM BigDye Terminator Cycle Sequencing Kit and an ABI 310 Genetic Analyzer (PE Biosystems) according to the manufacturer's protocol. DNA was extracted, amplified, cloned, and sequenced three different times to note any discrepancies that might arise during these procedures. Three clones were sequenced of each strain to ensure the cultures were pure, and to again note any discrepancies that might exist within the sequences. Additionally, PCR products were sequenced directly to determine whether or not a single product was present. Both strands of clones JW/KA-1, JW/KA-2^T, and JW/JH-Fiji-2 were completely sequenced using a suite of 16S rRNA-specific primers (Vetriani et al. 1999) to generate an overlapping set of sequences which were assembled into one contiguous sequence. Sequence alignments were performed using the software suite ARB (Ludwig et al. 2004). Phylogenetic analysis was performed using 1,307 homologous nucleotides. Using a subset of sequences, and based on manual sequence alignments and secondary structure comparisons, all nucleotides were used to construct distance matrices by pairwise analysis with the Jukes and Cantor correction (Jukes and Cantor 1969). Maximum-likelihood, maximum-parsimony and neighbor-joining analyses were done as previously described (Götz et al. 2002). The FastDNAm1 (Olsen et al. 1994) tree is presented, and the *T* value was optimized to *T* = 1.2. Bootstrap values were based on 1,000 trials.

DNA–DNA hybridization

DNA–DNA hybridization was performed by the German culture collection (DSMZ). DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970), with the modifications described by Huss et al. (1983), using a model Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with in situ temperature probe (Varian).

Nucleotide sequence accession numbers of novel isolates

The 16S rRNA gene sequences of strain JW/KA-2^T sequences A and B and JW/JH-Fiji-2 were deposited into GenBank under the accession numbers AF282253, AF282254, and AF282252, respectively. The sequence of strain R1^T was deposited as DQ095862.

Results

Morphology

As examined by phase contrast microscopy, the iron-reducing strains JW/KA-1, JW/JH-Fiji-2 and JW/KA-2^T cells were slightly curved rods, approximately 1.8 by 0.5 μm. They were usually found in V-shaped pairs, although chains were not uncommon. In mineral Fe-media, tumbling motility was observed during light microscopic imaging of cultures incubated under optimal growth conditions. Electron microscopy revealed the presence of 2–3 peritrichously located flagella (Fig. 1). Spores were never observed by phase contrast or electron microscopy. Cultures were unable to withstand 5 min. of heat treatment at 100°C, thus, heat resistant spores appeared to be absent. Cells stained Gram positive. Colonies formed on the agar surface in roll tubes (mineral medium with AQDS as electron acceptor) and were typically small (1–2 mm in diameter), flat, round, and white in color after 24 h.

The CO-oxidizing strain R1^T cells were short, straight rods, motile with peritrichous flagella. They divided by binary fission, and ultra-thin sections revealed a Gram-type positive cell wall (Wiegel 1981; Svetlichnyi et al. 1994).

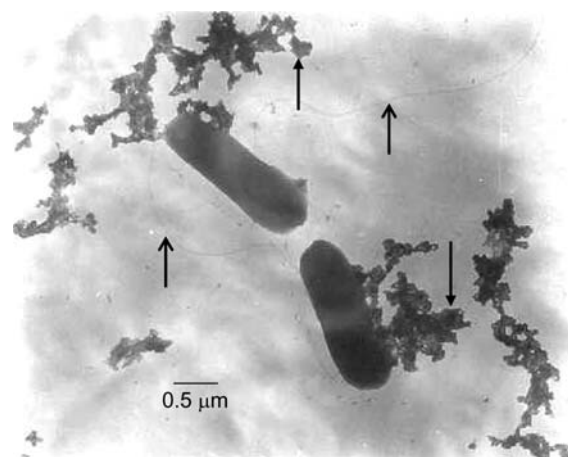


Fig. 1 Electron micrograph of JW/KA-2^T. Negative staining preparation using uranyl acetate. Arrow indicates flagella, filled triangle head arrow indicates Fe precipitation

Chemolithoautotrophic growth

The iron-reducing strain JW/KA-2^T and the CO-oxidizing strain R1^T were obligately anaerobic as O₂ concentrations of 0.2% in the gas phase were bactericidal. Although the relationship was not strictly proportional, an increase in cell number of strain JW/KA-2^T was observed with a concomitant increase in magnetite formation (Fig. 2a) during growth on CO₂/H₂ with Fe(III) as the electron acceptor. The amount of Fe(II) produced per cell in a growing culture of strain JW/KA-2^T was initially high, but steadily decreased as the cells entered the later phases of growth. Both resting cells and growing cells in the culture may have been responsible for Fe(III) reduction. Strain JW/KA-2^T utilized CO₂, yeast extract, casamino acids, fumarate, and crotonate in the presence of H₂. In the absence of H₂, JW/KA-2^T used formate as an electron donor and Fe(III) oxide/hydroxide, 9,10-anthraquinone 2,6-disulfonic acid, fumarate, and thiosulfate as terminal electron acceptors. None of the iron-reducing strains were able to grow on CO or a CO/H₂ mixture. In contrast, strain R1^T grew

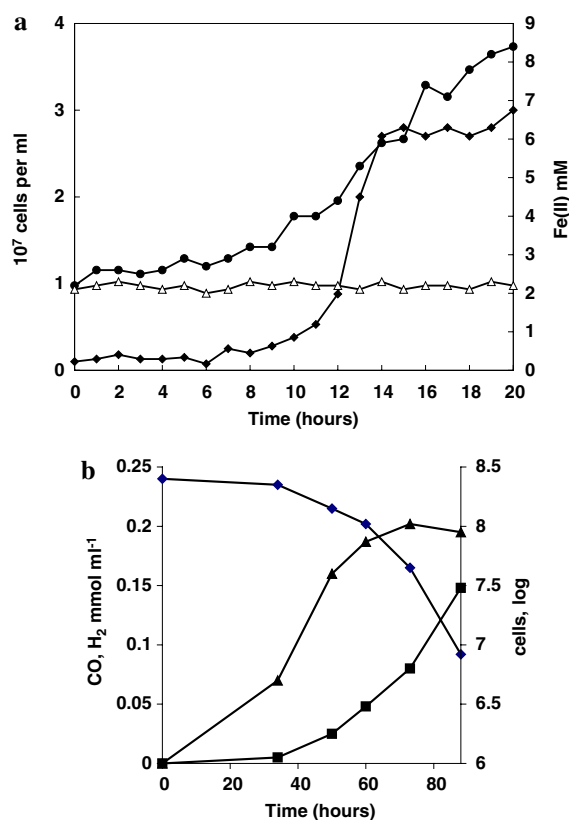


Fig. 2 Growth and **a** Fe(II) formation by JW/KA-2^T and **b** CO utilization/ H₂ production by strain R1^T. **a** Cell growth of strain JW/KA-2^T (diamond) paralleled Fe(II) accumulation (circle) when cells were grown in a mineral Fe medium at pH^{25C} 7.2 and 60°C as compared to a sterile control (triangle). Gas atmosphere was 80:20 H₂-CO₂ (v/v). **b** Cell growth of strain R1^T (triangle) was accompanied by CO utilization (diamond) and H₂ production (square) when cells were grown in a mineral medium at pH^{25C} 7.2 and 70–72°C. Gas atmosphere was 100% CO

chemolithoautotrophically on CO (Fig. 2b) and produced equimolar quantities of H₂ and CO₂. The minimal generation time was 1.3 h. However, strain R1^T did not grow with either Fe(III) citrate or Fe(III) oxide/hydroxide in mineral media supplied with HCO₃⁻, acetate, lactate, yeast extract, formate, or citrate under 100% H₂ or 100% CO atmosphere. Strain R1^T did not grow in media supplemented with AQDS, HCO₃⁻, acetate, lactate, yeast extract, formate, or citrate under 100% H₂, and did not reduce AQDS during growth on CO. Strain R1^T did not grow on yeast extract, formate, citrate, lactate or a H₂:CO₂ mixture in media supplemented with SO₄²⁻, S₂O₃²⁻, or elemental sulfur.

The temperature range for growth of the iron-reducing strain JW/KA-2^T at pH^{60C} 7.0 was 50–75°C (opt. 73–74°C) (Fig. 3a); no growth occurred below 47°C or above 77°C. The pH^{60C} range for growth of JW/KA-2^T was between 6.5 and 8.5 (opt. ~7.1–7.3) (Fig. 3b); no growth occurred below pH^{60C} 5.5 or above 9.0. Using a temperature of 72°C and a pH^{60C} of 7.3, the shortest doubling time observed was 46 min.

The temperature and pH ranges for magnetite production by resting cells of JW/KA-2^T were broader than those for growth. The temperature range (Fig. 3c) was from 50 to 90°C (opt. 74°C) and pH^{60C} range from 5.5 to 10.3 (opt. 7.3) (Fig. 3d).

Experiments to determine an optimal headspace to liquid medium ratio revealed a requirement for ~1.5 volumes of headspace for each volume of liquid. Large headspace volumes (100:1 and 200:1) resulted in no growth or iron reduction (data not shown). Experiments with varying levels of atmospheric H₂ indicated that 100% H₂ was optimal for both cell yields and Fe(III) reduction. However, growth was observed at H₂ concentrations as low as 0.1% (v/v) in the headspace.

The observed stoichiometry of iron reduction to H₂ uptake was 1.3 mol of Fe(II) produced per mol of H₂ utilized. The theoretical ratio is 2 mol of Fe(II) produced per 1 mol of hydrogen utilized, but some of the hydrogen was used in reduction of CO₂ to biomass (0.35 mol CO₂ reduced/mol Fe(III) reduced).

By refeeding the JW/KA-2^T cultures at 24 h intervals with H₂/CO₂ gas mixtures, a maximal cell yield of 10⁸ cells ml⁻¹ was obtained. The bacteria exhibited a high rate of Fe(III) reduction to Fe(II). For example, *Thermoterrabacterium ferrireducens* (Slobodkin et al. 1997), reduces Fe(III) to Fe(II) at a maximal rate of 0.6 μmol h⁻¹ ml⁻¹ in cultures containing 1.2 × 10⁹ cells. Under optimal growth conditions, strain JW/KA-2^T reduced approximately 1.3 μmol of Fe(III) to Fe(II) h⁻¹ ml⁻¹ in cultures containing 3.0 × 10⁷ cells.

Substrate utilization

In the presence of Fe(III) as an electron acceptor and H₂ as an electron donor, the iron-reducing strain JW/KA-2^T utilized CO₂, fumarate (20 mM), yeast extract (1%), casamino acids (1%), and crotonate (20 mM),

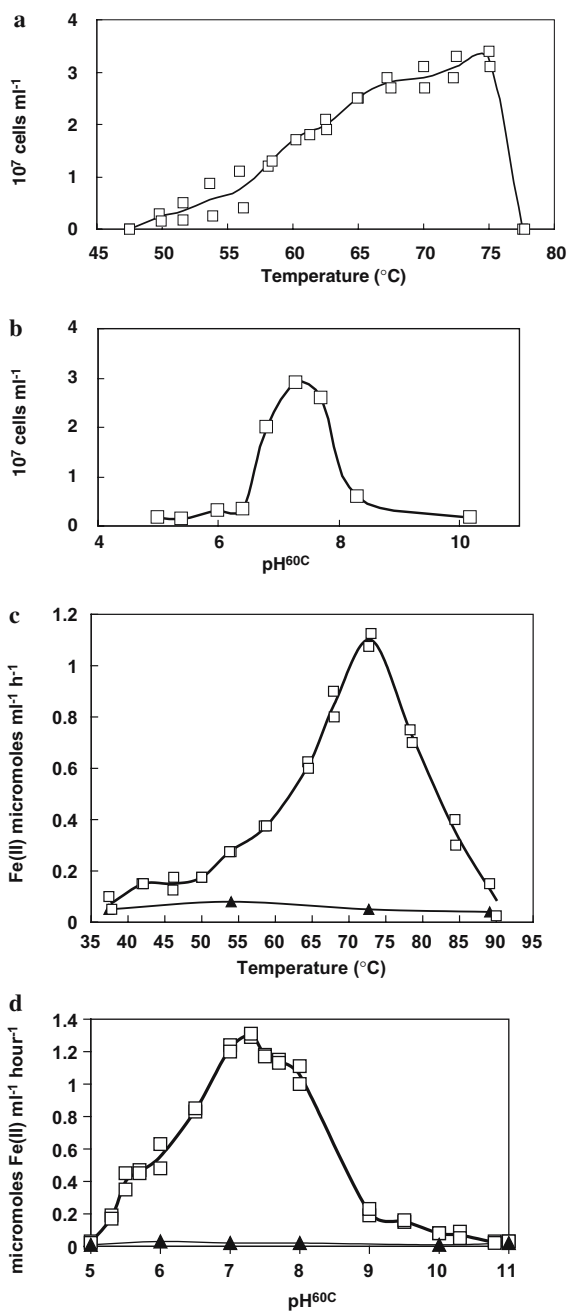


Fig. 3 Influence of incubation **a** temperature and **b** pH on growth of JW/KA-2^T. Influence of incubation **c** temperature and **d** pH on Fe(III) reduction by resting cells of JW/KA-2^T. **a** Fe-medium with a pH^{60C} 7.3 was inoculated with 1×10^6 cells/ml. After 24 h incubation with shaking (20 rpm), cells were counted using a Petroff-Hausser counting chamber. **b** Fe-medium was inoculated with 1×10^6 cells/ml and incubated at 72°C. After 24 h incubation with shaking (20 rpm), cells were counted using a Petroff-Hausser counting chamber. **c** Resting cells were inoculated to a concentration of 5×10^7 cells/ml, and incubated for 24 h in mineral media with a pH^{60C} of 7.2 under an 80:20 H₂-CO₂ (v/v) atmosphere. Fe(II) accumulation (*square*) was monitored and compared to a sterile control (*triangle*). A pH meter calibrated at 60°C was used to determine pH of the media. **d** Resting cells (*square*) were inoculated to a concentration of 5×10^7 cells/ml, incubated at 60°C with shaking (20 rpm). Fe(II) accumulation was monitored (*square*) as compared to a sterile control (*triangle*)

and (without the addition of H₂) formate (20 mM) as carbon sources, but no growth (with or without Fe(III)) on tryptone (1.0%), glucose (20 mM), galactose (20 mM), xylose (20 mM), sucrose (20 mM), propionate (20 mM), starch (5 g/l), sodium acetate (30 mM), succinate (20 mM), lactic acid (20 mM), glycerol (130 mM), cellobiose (20 mM), ethanol (20 mM), 1-butanol (20 mM), 2-propanol (20 mM), acetone (20 mM), phenol (10 mM), ethylene glycol (20 mM), 1,3 propanediol (20 mM), catechol (20 mM) or olive oil (10 ml/l) as carbon and energy sources. In the controls, glucose, sucrose, xylose, cellobiose, galactose, and starch reduced Fe(III) to Fe(II) abiotically at rates similar to those of a growing culture. However, no increase in cell numbers was observed with these substrates; i.e., growth was not observed with these particular substrates.

Electron acceptors

The oxidation of hydrogen gas by the iron-reducing strain JW/KA-2^T was coupled to the reduction of Fe(III) to magnetite and siderite, as determined by X-ray diffraction analysis (data not shown). Strain JW/KA-2^T also used AQDS (20 mM), thiosulfate (20 mM) and fumarate (20 mM) as electron acceptors but not ferric citrate, nitrate, sulfate (all at 20 mM), elemental sulfur (sublimated, 0.1% w/v), elemental sulfur (precipitated, 0.1% w/v), crystalline iron (III) oxide, 15 mM manganese (IV) added as MnO₂ (Kostka and Nealson 1998), 100 μM selenium (VI) added as selenium acetate, 10 μM uranium (VI) added as uranyl acetate, or 100 μM arsenic (V) added as potassium arsenate.

The CO-oxidizing strain R1^T did not use either Fe(III) or AQDS as electron acceptors under 100% H₂ and it did not reduce AQDS during growth on CO. Strain R1^T did not grow on CO in the presence of NO₃⁻, Fe(III) oxide/hydroxide, Fe(III) citrate, or SO₃²⁻. Strain R1^T grew on CO in the presence of SO₄²⁻, S₂O₃²⁻, or fumarate, though the presence of the previously mentioned electron acceptors did not stimulate the growth and no indication of the reduction of either SO₄²⁻, S₂O₃²⁻, or elemental sulfur in media supplemented with yeast extract, formate, acetate, pyruvate, citrate, succinate, lactate or H₂:CO₂, respectively, was observed. Strain R1^T also did not use either NO₃⁻, SO₃²⁻, or fumarate as electron acceptor while growing on H₂:CO₂ (66:34).

Antibiotic susceptibility

During growth at 60°C in mineral iron medium, strain JW/KA-2^T was resistant to tetracycline and ampicillin at 100 μg ml⁻¹ (higher concentrations not tested). It was resistant to 10 μg ml⁻¹ rifampicin, erythromycin, streptomycin and chloramphenicol but not at 100 μg ml⁻¹. The latter four were bactericidal at 100 μg ml⁻¹.

It should be noted that tetracycline and ampicillin lose their effectiveness rapidly at this incubation temperature (Peteranderl et al. 1990), so the exact concentration of these antibiotics JW/KA-2^T is resistant to is unclear. However, due to their bactericidal action they could still be used to grant some selective advantage to strain JW/KA-2^T in enrichment cultures. Strain R1^T grows with antibiotics as previously described (Svetlichnyi et al. 1994).

Lipid extraction and fractionation

The phospholipid fatty acid (PLFA) profile of strain JW/JH-Fiji-2, which showed 99% 16S rRNA gene sequence similarity to JW/KA-1 and JW/KA-2^T, contained the following fatty acids (mol%): i15:0 (32.2 ± 6.4), a15:0 (4.1 ± 0.9), i16:0 (0.8 ± 0.1), 16:0 (15.9 ± 1.0), i17:0 (35.0 ± 4.5), a17:0 (11.2 ± 1.5), and 18:0 (0.8 ± 0.2). The neutral lipid fraction of strain JW/JH-Fiji-2 contained the following respiratory quinones (mol%): demethylmenaquinone-9 (82.7) as the major compound, menaquinone (MK)-4 (2.2), MK-5 (0.7), MK-6 (2.2) and ubiquinone (UQ)-6 (9.0), UQ-7 (0.6), UQ-9 (1.4), UQ-10 (1.2) as minor compounds. The minor quinones, except UQ-9, were also found in the control (culture media) and could derive from passive accumulation in the membrane (R. Geyer, unpublished results).

G + C content

The mean (± SD from three measurements) guanosine (G) plus cytosine (C) content of the genomic DNA of strain JW/KA-2^T was 52.7 ± 0.3 mol% G + C as determined by HPLC (Mesbah et al. 1989). Strain R1^T DNA G + C content was 52 ± 1 mol% and was determined by melting-point analysis (Marmur and Doty 1962), using *Escherichia coli* K12 as a reference.

Phylogeny

DNA was extracted, amplified, cloned, and sequenced three different times from strains JW/KA-1, JW/KA-2^T, and JW/JH-Fiji-2. Three clones were sequenced for each of the above three listed strains to ensure that the cultures were pure, and to note any discrepancies that may exist within the sequences. Additionally, PCR products were sequenced directly to determine whether or not a single product was present. Sequencing of the PCR products indicated that there were two 16S rRNA gene sequences, designated A and B, present in both JW/KA-1 and JW/KA-2^T, i.e. each strain has two 16S rRNA gene sequences. The two strains differed slightly in the cell and colony morphology at the time of isolation. When comparing the two 16S rRNA gene sequences from one strain, a similarity of 98.9% was

observed with the changes distributed in the variable regions throughout the 16S rRNA gene sequence. The sequences were checked from three different colony isolations and three different DNA isolations of each strain after the strains were isolated nine times as a single colony in subsequent agar roll tube cultures. It should be noted here that comparable situations have been reported before, i.e. single-cell-derived pure cultures contained two different 16S rRNA gene sequences with up to 5% inferred differences in substitutions and bracketing different species (Amann et al. 2000; Onyenwoke et al. 2006). For strain JW/JH-Fiji-2, only one 16S rRNA gene sequence was observed. Further isolates need to be obtained and analyzed to test whether the occurrence of two 16S rRNA gene sequences is common among strains of this novel species.

The 16S rRNA gene sequences of strain R1^T and strains JW/KA-2^T, JW/KA-1 and JW/JH-Fiji-2 possessed 99% sequence similarity. The 16S rRNA gene sequences of JW/KA-2^T, JW/KA-1 and JW/JH-Fiji-2 possessed only low similarity to any other previously described thermophilic or mesophilic iron-reducers. The closest neighbors on the 16S rRNA gene sequence-based phylogenetic tree is *Thermanaeromonas toyohensis* (Fig. 4), as well as the *Desulfotomaculum* species, several of which are also capable of chemolithoautotrophic growth, and the acetogenic *Moorella* species, of which two are also able to grow chemolithoautotrophically with CO (Menon and Ragsdale 1996; J. Wiegel, unpublished results). The chemolithoautotrophic, iron-reducing strains do not produce acetate although they share with *Moorella* species a high (52–56) mol% G + C content.

DNA–DNA hybridization

DNA–DNA hybridization between strains R1^T and JW/KA-2^T was 35%.

Discussion

Iron-reducing strains. Physiologically, the three iron-reducing strains JW/KA-1, JW/KA-2^T, and JW/JH-Fiji-2 were different from other iron-reducing, thermophilic bacteria. They required H₂ or formate as their electron donor. They were not able to grow chemoorganotrophically. One of the distinguishing features of *Thermolithobacter ferrireducens* was that it only grew to low cell densities. However, the specific rates of Fe(III) reduction per biomass unit were approximately 10 times higher than those which have been reported for other iron-reducers (Table 2). The 16S rRNA gene sequence analysis has distinguished these strains from other thermophilic iron-reducers, such as *Bacillus infernus* (which is only capable of iron reduction under heterotrophic conditions) (Boone et al. 1995), *Thermoterrabacterium ferrireducens* (which utilizes glycerol, and

Fig. 4 Phylogenetic tree. Unrooted phylogenetic tree showing the position of *Thermolithobacter ferrireducens* strains JW/KA-2^T, with sequences A and B, and JW/JH-Fiji-2, and *T. carboxydivorans* strain R1^T. Two 16S rRNA gene sequences were also observed for strain JW/KA-1. The tree was generated by maximum-likelihood. Reliability values at each internal branch are percentages obtained from 1,000 trials. The scale bar represents the expected number of changes per sequence position. *Thermus aquaticus* was used as outgroup. The superscript *T* denotes that the strain is the type strain of the species

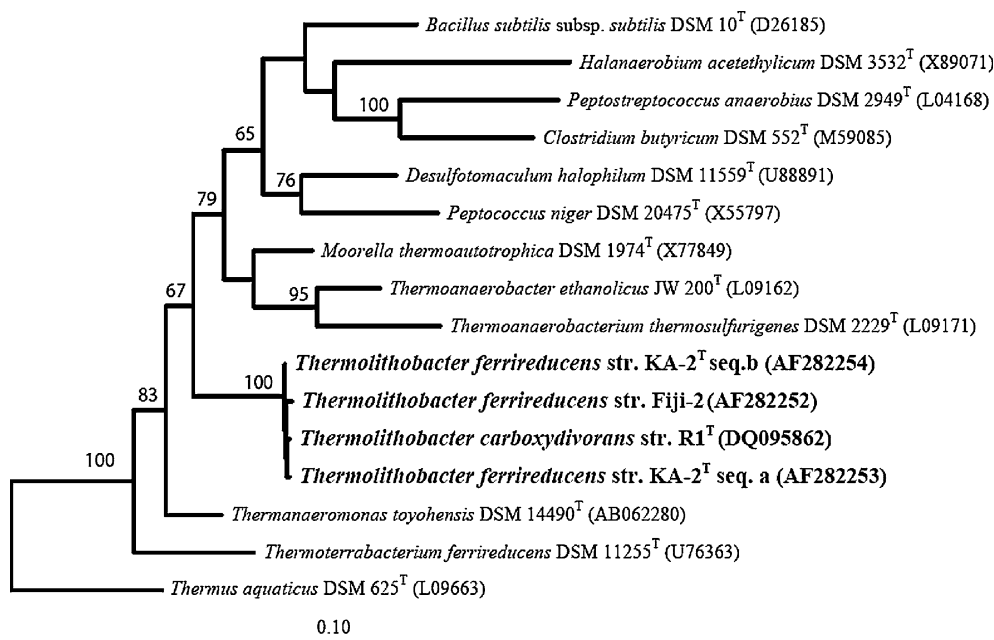


Table 2 A comparison of the rates of Fe(III) reduction by *Thermolithobacter ferrireducens* strain JW/KA-2^T to other iron-reducing bacteria

Microorganism (T_{opt}/T_{max} , °C)	Rate of Fe(III) reduction ($\mu\text{mol Fe(II) formed ml}^{-1} \text{ h}^{-1}$) at:	
	Exponential cell density	Extrapolated for 10^8 cells ml^{-1}
Thermophilic		
<i>Thermolithobacter ferrireducens</i> (73/75)	1.3 (3×10^7 cells/ml)	4.3
<i>Thermoterrabacterium ferrireducens</i> (65/74)	0.6 (6×10^8 cells/ml)	0.1
<i>Deferribacter thermophilus</i> (60/65)	0.1 (5×10^7 cells/ml)	0.2
<i>Thermoanaerobacter siderophilus</i> (70/78)	0.3 (8×10^7 cells/ml)	0.4
Mesophilic		
<i>Geobacter metallireducens</i> (33/40)	0.4 (2×10^8 cells/ml)	0.2

grows to higher densities in similar media) (Slobodkin et al. 1997), and *Thermoanaerobacter siderophilus* (which also utilizes sulfite and elemental sulfur as an electron acceptor) (Slobodkin et al. 1999). In agreement with the 16S rRNA gene sequence analyses, we propose that the strains JW/KA-1, JW/JH-Fiji-2 and JW/KA-2^T represent a novel genus and species, *Thermolithobacter ferrireducens*.

These microorganisms may also be interesting models for early life on Earth, and possibly extra-terrestrial life forms. Of special note in this regard are their ability to grow autotrophically in mineral medium, the use of Fe(III) as a terminal electron acceptor, and a high Fe(III) reduction rate. Presuming that a reservoir of Fe(III) could be provided, JW/KA-2^T would require little more than H₂, CO₂, trace minerals, and elevated temperatures from geothermal activity to thrive; i.e. as might be present in deep subsurface environments. Properties of *Thermolithobacter ferrireducens*, i.e. reducing Fe(III) in the range of pH^{60C} 5.5–10.3 and temperature ranges of 50–90°C, indicate that this or

closely related organisms could have contributed to geologically important iron reduction, such as forming the Precambrian Banded Iron Formations, especially since the resting cells showed an extended pH and temperature range and high Fe(III) reduction rates. Studies have indicated that, on Earth, ultra-fine grain magnetite is frequently the product of microbial Fe(III)-reduction (Gold 1992). Similar ultra-fine grains of magnetite have been observed in the Martian meteorite ALH84001 (McKay et al. 1996). It is of note to mention *Thermoterrabacterium ferrireducens* (Slobodkin et al. 1997) was isolated from a hot spring runoff at Calcite Spring a few meters away from the sample site from which strain JW/KA-2^T was isolated. However, the sampling site from which strain JW/JH-Fiji-2 was isolated is far apart, which suggests that this bacterium could be widespread in various geothermal environments (Slobodkin and Wiegel 1997).

CO-oxidizing strain. The CO-oxidizing strain R1^T was distinguished by 16S rRNA gene sequence analysis from any other known hydrogenogenic, CO-trophic

bacteria. Strain R1^T differed from *Carboxydothemus hydrogenoformans*, the type species of the genus to that strain R1^T was originally assigned, by the inability of strain R1^T to grow employing AQDS reduction and in the ability of strain R1^T to grow fermentatively on several carbohydrates while *C. hydrogenoformans* is unable to grow fermentatively on carbohydrates.

Novel lineage *Thermolithobacteria*. In spite of their differences, strain R1^T and strains JW/KA-1, JW/KA-2^T, and JW-Fiji-2 possessed sufficient 16S rRNA gene sequence similarity to assign them to the same genus. Taking into account that strains JW/KA-1, JW/KA-2^T, and JW-Fiji-2 only grow chemolithotrophically and strain R1^T prefers chemolithoautotrophic growth (cell yield during the growth on CO is 10 times higher than during the growth on glucose, data not shown), we propose *Thermolithobacter* as a novel genus within the phylum 'Firmicutes'.

The phylum 'Firmicutes' is divided into three classes: the 'Clostridia', Mollicutes, and 'Bacilli' (Garrity et al. 2002). Levels of 16S rRNA gene sequence similarity between the lineage containing the *Thermolithobacter*

and well-established members of the three classes of the 'Firmicutes', i.e. represented by the type species of the type genus of the 'Bacilli' and 'Clostridia', *Bacillus subtilis* and *Clostridium butyricum*, respectively, are less than 85%, and the *Thermolithobacter* appear to form their own distinct lineage apart from the members of the three existing classes (Fig. 5). Even though *Helio-bacterium chlorum* (a member of class 1) and *Alicyclobacillus acidocaldarius* (class 3) are the closest neighbors to the *Thermolithobacter* on the phylogenetic tree (Fig. 5), it should be noted that the classifications for these taxa are still tentative (Garrity et al. 2002). The 16S rRNA gene sequences of representative members of closely related (Schloss and Handelsman 2004) phylogenetic taxa outside the 'Firmicutes' were included in the tree (Fig. 5). The tree also shows the phylogenetic position of *Thermolithobacter* is indicative of a distinct lineage within the 'Firmicutes'. It is therefore proposed that the *Thermolithobacter* represent a new class within phylum BXIII 'Firmicutes', *Thermolithobacteria* (Gibbons and Murray 1978; Garrity et al. 2002).

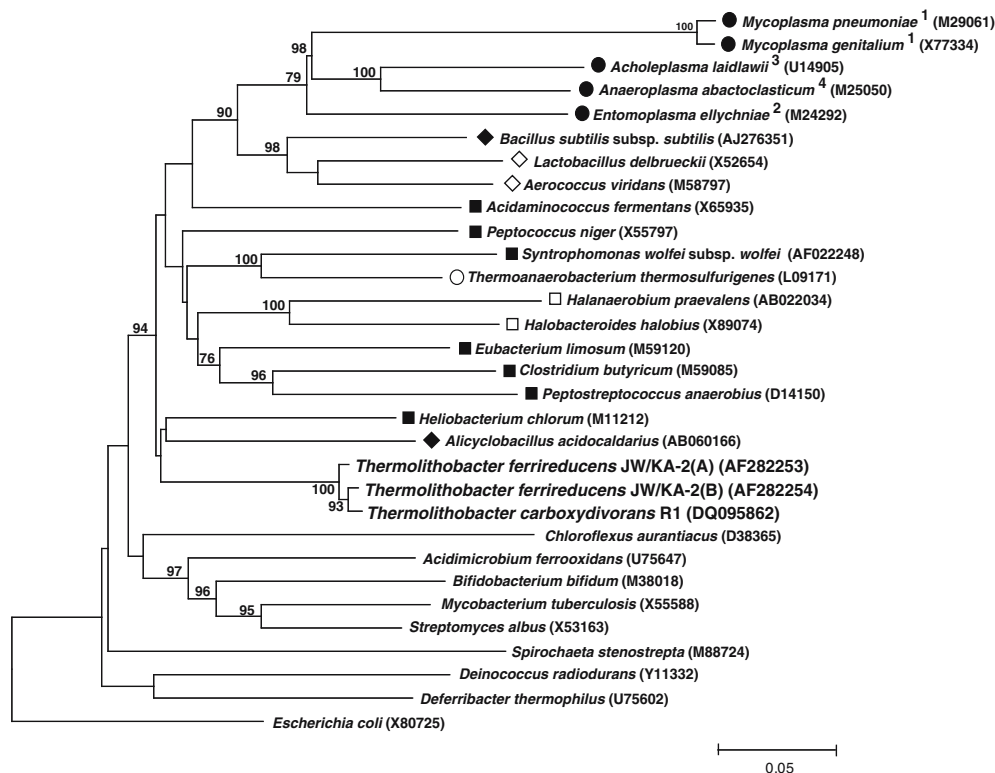


Fig. 5 Phylogenetic tree of higher taxa. Phylogenetic tree constructed from the 16S rRNA gene with maximum likelihood correction for synonymous changes using the neighbor-joining algorithm. GenBank accession numbers are indicated in parentheses. The three classes of the phylum 'Firmicutes' are indicated. For the 'Clostridia' (class 1), the order 1 *Clostridiales* are indicated by (filled square) while the order 2 'Thermoanaerobacteriales' and order 3 *Halanaerobiales* are indicated by (open circle) and (open square), respectively. The class 'Bacilli' is divided into the order 1 *Bacillales* (filled diamond) and order 2 'Lactobacillales' (open

diamond) while the members of the class *Mollicutes* are indicated by (filled circle) with the order indicated; i.e. ¹order 1 *Mycoplasmatales*, ²order 2 *Entomoplasmatales*, ³order 3 *Acholeplasmatales*, and ⁴order 4 *Anaeroplasmatales* (Garrity et al. 2002). To have a meaningful tree, each order is only represented by the type species of the type genus for the class and for the first family for each of the different orders. Numbers at nodes indicate bootstrap support values for 100 replicates with only values above 75 displayed on the tree. Scale bar denotes number of nucleotide substitutions per site. *Escherichia coli* was used as the outgroup

Description of *Thermolithobacteria* classis nov.

Thermolithobacteria (Ther.mo.li.tho.bac.te'ri.a. Gr. adj. *thermos*, hot; Gr. masc. n. *lithos*, stone; N. L. *bacter* masc. equivalent of Gr. neut. n. *baktron*, rod staff; suff. -*ia*, ending proposed by Gibbons and Murray (1978) to denote a class; N. L. neut. pl. n. *Thermolithobacteria*, thermophilic, lithotrophically growing rods).

Class of the 'Firmicutes'. Segregation of these organisms is warranted by: (1) their distinct phylogenetic lineage within the 'Firmicutes', and (2) their obligate, or preferred, chemolithotrophic growth. Description is the same as that of the genus. Type order: *Thermolithobacterales*.

Description of *Thermolithobacterales* ord. nov.

Thermolithobacterales (Ther.mo.li.tho.bac.ter'ales. N.L. masc. n. *Thermolithobacter*, the type genus of the order; suff. -*ales*, ending to denote order; N.L. fem. pl. n. *Thermolithobacterales*, the order of the genus *Thermolithobacter*).

Description is the same as that for the genus. Type family: *Thermolithobacteraceae*

Description of *Thermolithobacteraceae* fam. nov.

Thermolithobacteraceae (Ther.mo.li.tho.bac.te.ra'ce.ae. N.L. masc. n. *Thermolithobacter*, the type genus of the family; N.L. suff. -*aceae*, ending denoting a family; N.L. fem. pl. n. *Thermolithobacteraceae*, the family of *Thermolithobacter*).

Description is the same as that for the genus. Type genus: *Thermolithobacter*.

Description of *Thermolithobacter* gen. nov.

Thermolithobacter (Ther.mo.li.tho.bac'ter. Gr. adj. *thermos*, hot; Gr. masc. n. *lithos*, stone; N. L. masc. n. *bacter* equivalent of Gr. neut. n. *baktron*, rod staff; N. L. masc. n. *Thermolithobacter*, thermophilic lithotrophically growing rods). Cells are short rods. Gram-type positive cell wall. Bacterium. Obligate anaerobe. Thermophile. Neutrophile. Chemolithotroph. DNA G + C content is 52–53 ± 1 mol%. Type species: *Thermolithobacter ferrireducens*.

Description of *Thermolithobacter ferrireducens* sp. nov.

Thermolithobacter ferrireducens (fer.ri.re.du'cens. L.n. *ferrum*, iron; N.L. part. adj. *reducens*, leading back, bringing back, and in the chemistry converting to a different state; N. L. part. adj. *ferrireducens*, reducing iron (III)). Has the characteristics of the genus. Rod-shaped cells, approximately 1.8 by 0.5 µm, occurring in

singles, V-shaped pairs or chains. Exhibit tumbling motility via 2–3 peritrichously located flagella; spores not observed. Grows under anaerobic conditions in the presence of H₂/CO₂ in a temperature range of 50–75°C (opt. ~73°C). The pH^{60°C} range for growth is 6.5–8.5 (opt. 7.1–7.3). Uses as electron acceptor, Fe(III), 9,10-anthraquinone-2,6-disulfonic acid, thiosulfate, and fumarate. No growth occurs with sulfur (precipitated or sublimated), nitrate, sulfate, ferric citrate, crystalline ferric hydroxide, Mn(IV), U(VI), Se(VI) or As(V). Besides growing with H₂/CO₂, the species is able to grow in the presence of H₂ with casamino acids, yeast extract, fumarate, and crotonate, and also without the addition of H₂ with formate. Resistant to tetracycline and ampicillin at 100 µg/ml (higher concentrations not tested), and resistant to 10 µg/ml rifampicin, erythromycin, streptomycin and chloramphenicol but not at 100 µg/ml (all tests performed at 60°C in mineral iron medium). The latter four were bactericidal at 100 g/ml. The phospholipid derived fatty acids consist mainly (78%) of terminal branched species i15:0, i17:0, and a17:0 which is consistent with the affiliation to the Gram-type positive bacteria. The most abundant respiratory quinone species is demethylmenaquinone-9 (84%) if grown with yeast extract and H₂. The G + C content of the DNA of the type strain is 52.7 ± 0.3 mol%. Type strain is JW/KA-2^T (= ATCC 700985^T = DSM 13639^T), isolated from a mixed sample of geothermally heated sediment and water collected from a hot spring outflow at Calcite Spring in the Yellowstone National Park (Wyoming, USA).

Description of *Thermolithobacter carboxidivorans* sp. nov.

Thermolithobacter carboxidivorans (car.bo.xy.di.vo'rans. N.L. neut. n. *carboxydum* carbon monoxide; L. part. adj. *vorans* devouring, digesting; N.L. part. adj. *carboxidivorans*, digesting carbon monoxide) has the characteristics of the genus. Cells are short, straight rods, about 0.5 by 1–2 µm, arranged singly or in short chains. Cells are motile due to peritrichous flagella. On solid medium produces round, white, semi-translucent colonies, 1 mm in diameter. Grows within the temperature range from 40 to 78°C (opt. 70°C). The pH for growth ranges from 6.6 to 7.6 (opt. 6.8–7.0). Grows on CO autotrophically, producing hydrogen as the sole reduced product. Growth and CO consumption are inhibited by penicillin, erythromycin, and chloramphenicol but not streptomycin, rifampicin, or tetracycline (all 100 µg/ml). Does not reduce SO₄²⁻, S₂O₃²⁻, or fumarate during growth with CO. Does not reduce SO₄²⁻, S₂O₃²⁻, or elemental sulfur with yeast extract, formate, acetate, pyruvate, citrate, succinate, lactate or H₂:CO₂. Does not grow on a H₂:CO₂ mixture in the presence or absence of either NO₃⁻, SO₃²⁻, or fumarate. Does not grow on CO in the presence of Fe(III) citrate, Fe(III) oxide/hydroxide, or NO₃⁻. The G + C content of the DNA of the type

strain is 52 ± 1 mol%. The type strain is strain R1^T (= DSM 7242^T, = VKM 2359^T), isolated from a terrestrial hot spring at Raoul Island, Archipelago Kermadec.

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