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A thermophilic green sulfur bacterium from New Zealand hot springs, *Chlorobium tepidum* sp. nov. *

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Abstract. Thermophilic green sulfur bacteria of the genus *Chlorobium* were isolated from certain acidic high sulfide New Zealand hot springs. Cells were Gram-negative nonmotile rods of variable length and contained bacteriochlorophyll *c* and chlorosomes. Cultures of thermophilic chlorobia grew only under anaerobic, phototrophic conditions, either photoautotrophically or photoheterotrophically. The optimum growth temperature for the strains of thermophilic green sulfur bacteria isolated was 47–48° C with generation times of about 2 h being observed. The upper temperature limit for growth was about 52° C. Thiosulfate was a major electron donor for photoautotrophic growth while sulfide alone was only poorly used. N_2 fixation was observed at 48° C and cell suspensions readily reduced acetylene to ethylene. The G + C content of DNA from strains of thermophilic chlorobia was 56.5–58.2 mol% and the organisms positioned phylogenetically within the green sulfur bacterial branch of the domain Bacteria. The new phototrophs are described as a new species of the genus *Chlorobium, Chlorobium tepidum.*

Key words. *Chlorobium tepidum* – Photosynthesis – Green sulfur bacteria – Chlorosomes – Bacteriochlorophyll – Thermophily – Hot springs – 16S rRNA

Thermophilic phototrophic prokaryotes are the major components of laminated microbial mats that develop in the outflow waters of hot springs worldwide (Brock 1978; Castenholz 1969). In oxygenic microbial mats, either unicellular or filamentous cyanobacteria (or both) are present in the upper mat layers, usually in association with the filamentous green bacterium *Chloroflexus aurantiacus* (Bauld and Brock 1973; Pierson and Castenholz 1974a), and in some mats, with the filamentous bacteriochlorophyll *a*-containing phototroph *Heliothrix oregonensis* as well (Pierson et al. 1984; 1985). Thermophilic nonsulfur purple bacteria are also present in certain oxygenic microbial mats (Resnick and Madigan 1989). By contrast, anoxygenic microbial mats have been described from certain springs in which relatively high sulfide concentrations prevent growth of cyanobacteria, and hence, oxygen production (Castenholz 1973; Giovannoni et al. 1987; Ward et al. 1989). Such springs are typically pH neutral and average 30–60 μM in sulfide (Castenholz 1977; Ward et al. 1989). Anoxygenic mats can contain nearly pure stands of *Chloroflexus* (Giovannoni et al. 1987) or mixtures of *Chloroflexus* and the thermophilic purple sulfur bacterium *Chromatium tepidum* (Madigan 1984; 1986). Elemental sulfur is abundant in anoxygenic mats due to phototrophic oxidation of sulfide, and globules of elemental sulfur can be found attached to the outside of cells of *Chloroflexus* (Madigan and Brock 1975; Giovannoni et al. 1987) or within cells of *Chromatium* (Madigan 1984; 1986). Strains of *Chloroflexus* isolated from anoxygenic mats (designated GCF strains) are dark green in color, contain bacteriochlorophyll *c*, and are obligately phototrophic, differing in the latter connection from strains of *C. aurantiacus* isolated from oxygenic mats, all of which retain the ability to grow aerobically in darkness (Pierson and Castenholz 1974a, b). Nevertheless, GCF strains of *Chloroflexus* are filamentous bacteria, morphologically similar to *Chloroflexus* strains isolated from oxygenic mats (Giovannoni et al. 1987).

In the central volcanic area of North Island, New Zealand, unlaminated anoxygenic mats containing a rod-shaped green bacterium have been found in the outflow of certain hot springs (Castenholz 1988; Castenholz et al. 1990; Ward et al. 1989). Springs in which these mats develop are characteristically acidic (pH 4.5–6), contain high sulfide levels (0.2–2 mM), and have temperatures in the range of 45–55° C (Castenholz 1988; Castenholz et al. 1990). From samples of three such springs we have isolated pure cultures of rod-shaped green sulfur bacteria

* This paper is dedicated to Professor Norbert Pfennig on the occasion of his 65th birthday

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capable of growth up to 52° C (optimum ca 47–48° C). These organisms contain bacteriochlorophyll *c* and chlorosomes and are phylogenetically related to mesophilic green sulfur bacteria. However, because of a number of novel properties associated with these new anoxygenic phototrophs, including in particular their thermophilic character and lipid composition, we describe these organisms herein as a new species of the genus *Chlorobium, Chlorobium tepidum.*

Materials and methods

Source of the new organism

All strains of thermophilic chlorobia were isolated from microbial mats that developed in acidic high sulfide New Zealand hot springs. The location of all springs is detailed in Castenholz et al. (1990). The type strain of *Chlorobium tepidum* is strain TLS (Travelodge Stream; ATCC 49652). Two additional strains, designated S. PTE.-1 and GVP, were also obtained in pure culture by repeated application of the agar shake tube technique.

Media

All strains were routinely grown in maintenance medium, which was a modified version of Pfennig's medium consisting of (per liter of deionized water): EDTA, 10 mg; $MgSO_4 \cdot 7\ H_2O$, 0.2 g; $CaCl_2 \cdot 2\ H_2O$, 50 mg; NaCl, 0.4 g; KH_2PO_4, 0.5 g; NH_4Cl, 0.4 g; ammonium acetate, 0.5 g; $Na_2S_2O_3 \cdot 5\ H_2O$, 1.0 g; trace elements (EDTA, 5.2 g; $CoCl_2 \cdot 6\ H_2O$, 190 mg; $MnCl_2 \cdot 4\ H_2O$, 0.1 g; $FeCl_2 \cdot 4\ H_2O$, 1.5 g; H_3BO_3, 6 mg; $CuCl_2 \cdot 2\ H_2O$, 17 mg; $Na_2MoO_4 \cdot 2\ H_2O$, 188 mg; $NiCl_2 \cdot 6\ H_2O$, 25 mg; $ZnCl_2$, 70 mg; $VoSO_4 \cdot 2\ H_2O$, 30 mg; $Na_2WO_4 \cdot 2\ H_2O$, 2 mg; $NaHSeO_3$, 2 mg; deionized water, 1 l), 1 ml; vitamin B_{12}, 20 µg; $NaHCO_3$, 2 g; $Na_2S \cdot 9\ H_2O$, 0.6 g. Bicarbonate was autoclaved as a dry powder which after cooling was dissolved in sterile deionized water and the solution bubbled aseptically with pure CO_2 for twenty minutes before being added to the basal salts. Crystals of $Na_2S \cdot 9\ H_2O$ were washed in deionized water, dried, and then dissolved in boiling water and autoclaved immediately. After addition of sulfide to the sterile salts/bicarbonate/acetate medium, the pH was adjusted to 6.9 and the medium immediately dispensed into completely filled 17 ml screw-cap tubes or 250 ml bottles.

Growth conditions

Freshly inoculated cultures were placed in the dark for 2–3 h before being placed in the light (5400 lux, incandescent lamps). Cultures were grown in water baths at 47° C or in an incubator room at 44–48° C.

Bacteriochlorophyll and protein analyses

Absorption spectra were performed using an Hitachi U-2000 double-beam spectrophotometer. Spectra of intact cells were performed by suspending a few drops of an overnight culture in 60% sucrose. Extractions were performed by treating cell pellets with ice-cold methanol for 30 min in darkness, centrifuging at 27000 × g for 20 min and running spectra on the supernatant. Bacteriochlorophyll *c* concentrations were determined from methanol extracts at 669 nm using the extinction coefficient 86 $l \cdot g^{-1} \cdot cm^{-1}$ and bacteriochlorophyll *d* at 656 nm using the extinction coefficient of 82.3 $l \cdot g^{-1} \cdot cm^{-1}$ (Stanier and Smith 1960). Protein determinations were done using a dye-binding assay (Bulletin 1069; Bio-Rad Laboratories, Richmond, CA).

Isolation of DNA and determination of the mol% G + C content

The Marmur (1961) procedure was found to give inconsistent DNA yields from thermophilic chlorobia, thus a modified method was developed for obtaining high yields of genomic DNA. Cells (0.2–0.4 g wet weight) were suspended in 5 ml saline-EDTA buffer (0.15 M NaCl, 0.01 M EDTA, pH 8.0) and lysed by the rapid alkaline method of Beiji et al. (1987). The lysate was centrifuged for 5 min at 30590 × *g* and both the pellet and supernatant phases were treated with 1 mg of RNase for 45 min at 50° C, and then with 300 mg proteinase K overnight at 37° C. Following a single chloroform extraction, DNA was spooled with ice-cold ethanol and dissolved in 20 ml of 0.01 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M trisodium citrate pH 7). NaCl (1 M) was added to a final concentration of 0.1 M, and the DNA was reprecipitated with an equal volume of cold isopropanol. The spooled DNA was dissolved in 0.1 × SSC to give an A_{260} of 0.2–0.4, and the T_m determined from the melting profile using an Hitachi U-2000 spectrophotometer equipped with a thermoelectric cell holder. DNA purified from *Micrococcus luteus* (72% G + C), *Escherichia coli* (50% G + C), Calf thymus (43.2% G + C), and *Clostridium novyii* (29% G + C) were used as standards.

16S rRNA sequencing

RNA was isolated and the 16S rRNA sequenced by methods previously described (Brosius et al. 1978; Weisburg et al. 1989). Sequencing employed the dideoxynucleotide chain termination method (Sanger et al. 1977; Biggin et al. 1983) adapted for direct rRNA sequencing using reverse transcriptase (Lane et al. 1985; Weisburg et al. 1989). The 16S rRNA sequence of *C. tepidum* has been deposited in GenBank under the accession number M58468. It is more than 90% complete. Sequences were aligned in a sequence editor against a representative collection of bacterial 16S rRNAs (Woese et al. 1985), the pairwise evolutionary distances computed from percent similarities using the Jukes and Cantor (1969) correction as modified by G. J. Olsen (see Weisburg et al. 1989). The phylogenetic tree was computed from evolutionary distances by the method of De Soete (1983).

Nitrogenase

In vivo nitrogenase activities were determined by acetylene reduction as described by Madigan et al. (1984), except that the headspace in serum vials was N_2 instead of argon, light intensities for assays were approximately 5000 lux, and assays were run at 47–48° C. Nitrogen-fixing cultures of *C. tepidum* were grown in maintenance medium in which sodium acetate was substituted for ammonium acetate, NH_4Cl was deleted, sulfide was increased to 0.9 g $Na_2S \cdot 9\ H_2O$/liter, and 0.5 mM L-cysteine was added. Cells were grown on N_2 in stirred serum vials containing a headspace of N_2.

Electron microscopy

Cells were fixed by the method of Ryter et al. (1958), dehydrated in an ethanol series, and embedded in Spurr low-viscosity embedding medium. Sections were stained with uranyl acetate and lead citrate and micrographs taken on a Philips model 1300 electron microscope operating at 60 kV.

Chemicals

All chemicals used were of reagent grade and were obtained from either Fisher Scientific or Mallinckrodt Chemicals. RNase and proteinase K were obtained from Sigma; $Na_2S_2O_3 \cdot 5\,H_2O$ was obtained from Fisher.

Results

Enrichments and isolation

Several strains of a new thermophilic rod-shaped green bacterium were isolated from mat samples collected from high sulfide hot springs located in the central volcanic area of North Island, New Zealand (Castenholz 1988; Castenholz et al. 1990). Springs yielding green bacteria contained high levels of sulfide (0.2 – 2 mM) and were of relatively low pH (4.3 – 6.0). One such mat environment, named "Travelodge Stream" (Sulphur Bay, Rotorua, see Castenholz et al. 1990), consisted of a claylike channel covered with a dark green layer of unicellular bacteria. Core samples taken from Travelodge Stream showed a thick but relatively loose unlaminated dark green bacterial mat covered with a yellowish surface precipitate of elemental sulfur. The temperature of Travelodge Stream was about 45° C over the *Chlorobium* mat and the source water contained about 1 mM sulfide at pH 5.9 (Castenholz 1988; Castenholz et al. 1990).

Nonmotile green rod-shaped bacteria were observed microscopically from samples of the mat, and absorption spectra of mat material suggested the presence of organisms containing bacteriochlorophyll *c*. Enrichment cultures established with mat material from Travelodge Stream in a sulfide/CO_2 medium (Madigan 1986) containing 0.01% yeast extract and vitamin B_{12} and incubated at 45° C in 1000 lux incandescent light, yielded dark green cell suspensions within 48 h. The same medium supplemented with acetate (maintenance medium, see Methods) and inoculated with Travelodge Stream mat material yielded brilliant red cell suspensions, which upon subsequent isolation and purification proved to be a *Chromatium* species, probably related to *Chromatium tepidum* (Madigan 1986). However, from enrichments established in maintenance medium using inocula from two other New Zealand hot springs mats, additional strains of green bacteria were obtained. The strains were designated TLS (Travelodge Stream), GVP, and S.PTE.-1. Strain S.PTE.-1 (Sulfur Point) came from a hot spring (~ 50° C, pH 5.8) located at Sulfur Point-1 on the shore of Lake Rotorua, whereas strain GVP (Government Vent Pool) was from a spring-fed pond (~ 30° C, pH 6.1) at Government Gardens, Rotorua (Castenholz et al. 1990). In shake tubes, colonies of all strains had clearly defined margins. Colonies of strains TLS and GVP were intensely dark green in color; colonies of strain S.PTE.-1 were light green to yellow green.

Morphology and photosynthetic pigments

Individual cells of hot spring green bacteria were nonmotile, rod shaped to slightly curved, and sometimes grew in short chains (Fig. 1). Cells of strain TLS stained Gram-negative, with average dimensions of 0.6 – 0.8 × 1.3 – 2.6 μm (Fig. 1a), slightly larger than typical mesophilic *Chlorobium* species (Pfennig and Trüper 1989). Cells of strains S.PTE.-1 and GVP were about the same width but were significantly longer than cells of strain TLS and in some cases resembled short filaments (Fig. 1b). In overnight cultures cells of strain GVP measured 0.7 – 0.8 × 8.5 – 25 μm and of strain S.PTE.-1 0.6 – 0.8 × 3.2 – 11.7 μm.

Electron micrographs of thin sections of cells of strain TLS (Fig. 2) revealed the presence of chlorosomes, the structures which house light-harvesting pigments in green bacteria (Cohen-Bazire and Sistrom, 1966; Olson 1980). The chlorosomes of strain TLS measured 100 – 180 × 40 – 60 nm, within the range described for chlorosomes of other green bacteria (Schmidt 1980). During phototrophic growth of strain TLS, elemental sulfur globules appeared outside the cells in the early stages of growth; sulfur globules were both attached to cells and free-floating in the medium (data not shown). After about 4 – 6 h of phototrophic growth, sulfur globules disappeared and cultures reached a maximum density about 18 h later (at 47° C, see below).

Densely grown cultures of strain TLS were intensely dark green in color. Absorption spectra of intact cells showed maxima at 753 and 460 nm, corresponding to bacteriochlorophyll (bchl) *c* and the major carotenoid, chlorobactene, respectively (Fig. 3). Extraction of cells with methanol revealed a minor peak at 771 nm, reflecting the presence of small amounts of bchl *a*, as well as major peaks at 669 and 436 nm, reflecting the much higher concentrations of bchl *c* and chlorobactene, respectively (Fig. 3). Strains S. PTE.-1 and GVP showed essentially identical absorption properties as for strain TLS. Although cultures of strain TLS appeared more intensely green in color than cultures of typical mesophilic chlorobia grown to similar cell densities, the specific bchl *c* content of cells of strain TLS grown at low light intensities (≈ 500 lux) was about the same as the specific bchl *d* content of a mesophilic control organism, *Chlorobium limicola (vibrioforme)* f. *thiosulfatophilum* strain 8327 grown under the same light conditions (data not shown). However, specific carotenoid content determinations were not made in either organism and it is possible that elevated levels of chlorobactene in strain TLS may account for the visual differences in color between the two cultures.

Temperature relationships

Until the present isolations, the upper temperature limit for growth of chlorobia was about 35° C (Pfennig and Trüper 1989). By contrast, *Chlorobium* strain TLS grew up to 51° C, with optimal growth (generation time approx. 2 h) occurring at 47° C (Fig. 4). At 51° C, cells of strain TLS appeared normal microscopically, although final cell yields were lower than those obtained at the temperature optimum. No growth of strain TLS was observed below about 32° C or above 51 – 52° C. Similar

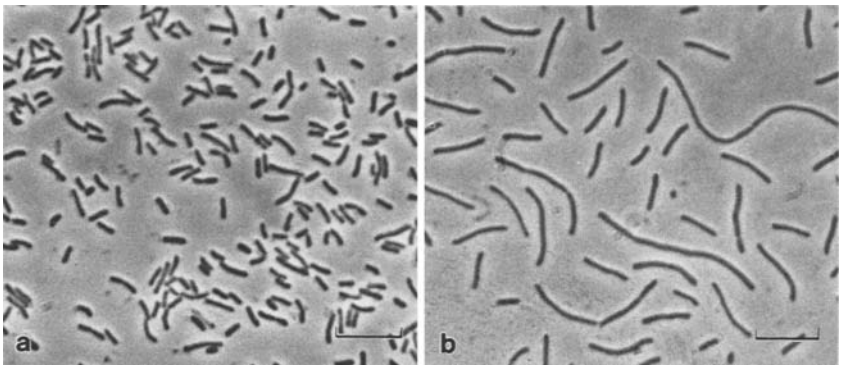

Fig. 1a, b. Phase contrast photomicrographs of cells of *Chlorobium tepidum* strains **a** TLS and **b** S. PTE.-1 Cultures were grown at 47° C for 48 h at 5400 lux. Marker bar = 5 μm

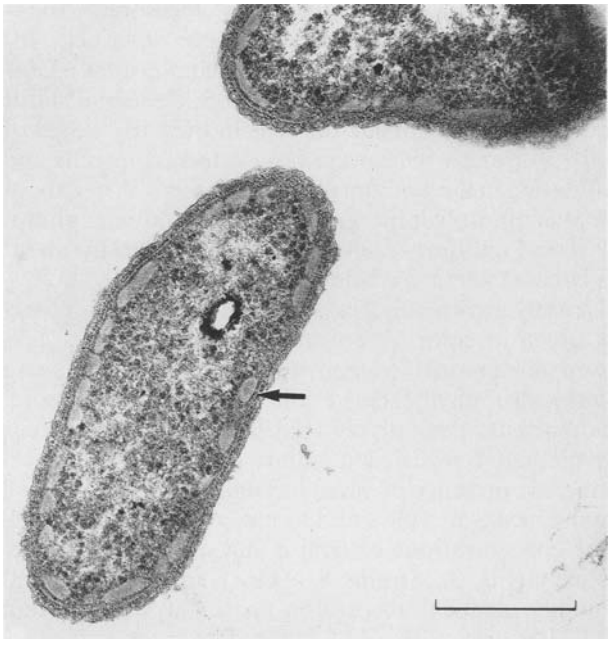

Fig. 2. Electron micrograph of thin sections of cells of *Chlorobium tepidum* strain TLS. Note the presence of chlorosomes (*arrow*) Marker bar = 0.5 μm

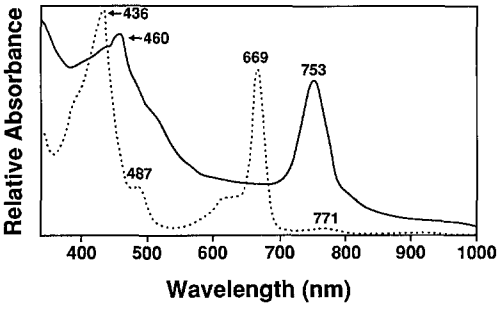

Fig. 3. Absorption spectra of intact cells (*solid line*) and methanol extracts of cells of *Chlorobium tepidum* strain TLS

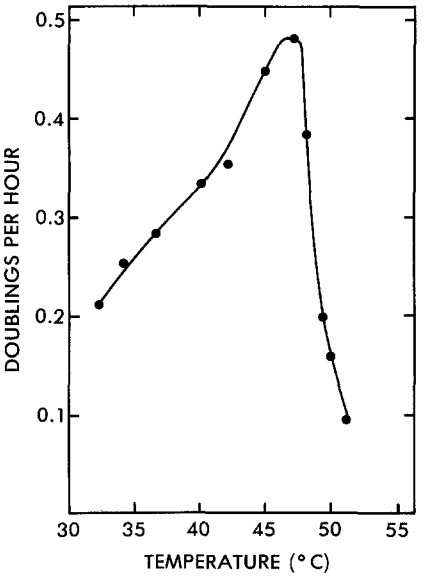

Fig. 4. Growth rate as a function of temperature for *Chlorobium tepidum* strain TLS. Cells were grown in maintenance medium at a light intensity of approximately 5000 lux

temperature profiles were obtained for strains GVP and S. PTE.-1. In contrast, the mesophilic *Chlorobium* strain 8327, used as a control in these experiments, had an optimal growth temperature of about 32° C but continued to grow up to about 40° C (data not shown). However, near the temperature limit for strain 8327 copious elemental sulfur production was observed and cells became slimey and settled out to the bottom of the tube. At 47° C, strain TLS grew in even suspension and only tended to settle out in old cultures. Under optimal growth conditions, cultures of *Chlorobium* strain TLS gave cell yields of approximately 0.5 – 0.6 mg/ml (dry weight) in under 24 h.

Nutritional and other properties

Growth of thermophilic chlorobia only occurred under anaerobic phototrophic conditions. Although sulfide was absolutely required for growth, sulfide alone was not a particularly good photosynthetic electron donor (Table 1). Cell yields of strain TLS grown either photoautotrophically on sulfide/CO_2 or photoheterotrophically on sulfide/CO_2/acetate (or pyruvate) were consistently quite low (Table 1). The upper limit of sulfide tolerance for strain TLS was about 4 mM and a minimum of about 1 mM sulfide was necessary for growth under any conditions. Addition of thiosulfate greatly stimulated growth

Table 1. Cell yields of *Chlorobium tepidum* strain TLS obtained in media containing various electron donors[a]

Electron donor[b]	Cell yield[c]
Sulfide (2.5 mM)	90
Sulfide (4 mM)	45
Sulfide (2.5 mM)	
plus 2 mM $S_2O_3^{2-}$	320
plus 4 mM $S_2O_3^{2-}$	700
plus 8 mM $S_2O_3^{2-}$	1000
plus 7.5 mM acetate	250
plus 7.5 mM acetate and 2 mM $S_2O_3^{2-}$	450
plus 7.5 mM pyruvate	200
plus 7.5 mM pyruvate + 2 mM $S_2O_3^{2-}$	360
plus 2 mM SO_3^{2-}	103

[a] Incubation temperature, 47–48° C
[b] Mineral salts/bicarbonate medium (pH 6.9) containing the listed additions
[c] Photometer units. 100 photometer units was equivalent to about 0.145 mg/ml dry weight of cells

and was absolutely required to obtain high cell yields (Table 1). In fact, the cell yield obtained in growth experiments was more dependent on the initial thiosulfate concentration than on the initial sulfide concentration, suggesting that although sulfide was required by strain TLS, perhaps as a source of sulfur for biosynthetic needs, it was not a prime photosynthetic electron donor. Sulfite could not substitute for thiosulfate as a photosynthetic electron donor (Table 1).

All three strains of thermophilic chlorobia were screened for their ability to photoassimilate various carbon sources. The general conclusion reached was that thermophilic chlorobia are not very nutritionally diverse. Of the organic compounds tested, stimulation of phototrophic growth was observed only with acetate and pyruvate (media contained growth-limiting levels of thiosulfate, see Table 1). No stimulation of growth was obtained from the following carbon sources: lactate, malate, propionate, butyrate, succinate, citrate, fumarate, ribose, fructose, glucose, lactose, asparagine, proline, threonine, serine, aspartate, glutamate, methionine, tryptophan, phenylalanine, valine, alanine, arginine, casamino acids, and yeast extract. Lack of growth on propionate was of particular interest, as this fatty acid commonly supports photoheterotrophic growth of mesophilic chlorobia (Pfennig and Trüper 1989).

Ammonia served as a nitrogen source for all three strains of thermophilic chlorobia. Glutamine was also used as a nitrogen source, but attempts to grow all strains on low levels of urea or nitrate as a nitrogen source were unsuccessful. Likewise, glutamate or aspartate did not serve as nitrogen sources. However, nitrogen fixation (acetylene reduction) assays of strain TLS indicated that this organism produced nitrogenase and is thus a nitrogen-fixing phototroph. Significant rates of acetylene reduction occurred at 47–48° C in cells of strain TLS grown on N_2 (~5 μmol ethylene produced · h^{-1} · mg^{-1} cell dry weight). Nitrogenase activity in strain TLS was totally repressed by growth on excess ammonia (data not shown).

Despite originating from an acidic hot spring, strain TLS grew best in culture at pH 6.8–7. Media adjusted to pH 6.4–6.5 (2.5 mM sulfide) and inoculated heavily grew only after an extended lag period, but media adjusted to pH 5.8 (2.5 mM sulfide) did not support growth. Slow growth did occur at pH 5.9 in media containing low (~1 mM) levels of sulfide (data not shown). Alkaline conditions were severely detrimental, as media adjusted to pH 7.3 or above failed to support growth of any strain of thermophilic *Chlorobium*. In media at pH 7 to which high levels (8–10 mM) of thiosulfate were added, extremely dense cultures (greater than 10^{10} cells/ml) were obtained that eventually turned from bright green to brown in color in the later stages of growth. This was probably due to the oxidation of thiosulfate to H_2SO_4 because the pH in such cultures dropped to as low as 4, presumably causing the conversion of bacteriochlorophyll to bacteriopheophytin. Cultures reaching these high densities and turning brown showed poor viability upon subculture.

Genetic properties

The DNA base composition (by thermal denaturation) of strain TLS was 56.5% G + C, well within the range for other members of the genus *Chlorobium* (49.0–58.1%, Pfennig and Trüper 1989), and very close to that of *Chlorobium* strain 8327 (56.1%). Strain GVP had a DNA base ratio identical to that of strain TLS. The G + C base ratio of strain S.PTE.-1, however, was slightly higher, 57.5–58.2 mol%.

Ribosomal RNA sequencing clearly placed the new organisms within the green sulfur bacterial branch of the eubacteria (Bacteria, Woese et al. 1990a, b). Comparison of 16S rRNA sequences between strain TLS and the mesophilic *Chlorobium* strain 8327 showed approximately 96% sequence homology (Table 2). In addition, a phylogenetic distance tree showed the thermophilic species to group more closely to strain 8327 (a thiosulfate-utilizer) than to the non-thiosulfate-utilizing *Chlorobium* species, *C. vibrioforme* (Fig. 5). As for other *Chlorobium* species, a large evolutionary distance exists between *C. tepidum* and *Chloroflexus* or between *C. tepidum* and purple phototrophic bacteria, while species of *Chlorobium* cluster rather tightly (Table 2 and Fig. 5).

Discussion

Until the discovery and culture of thermophilic green bacteria, the only green bacterium known capable of growth above 35–40° C was *Chloroflexus aurantiacus* (Pierson and Castenholz 1974a, b). It is a misnomer, however, to refer to *Chloroflexus* as a "green bacterium", since all evidence indicates that the Chlorobiaceae and Chloroflexaceae represent two widely divergent evolutionary lines, despite close similarities in the nature and housing of light harvesting pigments (Gibson et al. 1985; Pierson and Castenholz 1991). The present phylogenetic

Table 2. Evolutionary distance matrix for a collection of bacterial 16S rRNA sequences including *Chlorobium tepidum* (see Methods). Only positions represented by a known nucleotide in all sequences in the alignment are considered in the analysis. The 16S rRNA sequences are from the following sources: *Anacystis(A.) nidulans* (GenBank/EMBL accession no. X03538), *Bacillus(B.) subtilis* (Green et al. 1985), *Bacteroides(Ba.) fragilis* (GenBank/EMBL accession no. M11656) *Escherichia(E.) coli* (M24828), *Flexibacter(Fx.) flexilis* (M28056), *Chlorobium(C.) limicola (vibrioforme) f. thiosulfatophilum* (M31769), *Chlorobium(C.) vibrioforme* (M27804), *Chlorobium(C.) tepidum* (M58468), *Chloroflexus(Ch.) aurantiacus* (M34116), *Rhodobacter(Rb.) capsulatus* (M34129), and *Thermotoga(T.) maritima* (M21774)

	1	2	3	4	5	6	7	8	9	10
1. *C. tepidum*										
2. *C. limicola*	3.2									
3. *C. vibrioforme*	8.0	7.3								
4. *Fx. flexilis*	29.6	30.3	32.2							
5. *Ba. fragilis*	32.0	31.3	32.4	25.9						
6. *Ch. aurantiacus*	31.8	32.5	33.3	37.1	37.3					
7. *T. maritima*	30.3	28.7	30.0	34.1	33.8	30.4				
8. *B. subtilis*	25.9	26.3	27.3	32.2	31.8	29.4	26.1			
9. *A. nidulans*	28.7	28.5	27.4	32.2	35.6	32.6	29.8	22.6		
10. *Rb. capsulatus*	26.7	27.1	29.2	34.2	32.9	32.7	29.1	25.6	29.2	
11. *E. coli*	28.2	28.8	30.5	33.5	33.6	32.1	30.7	24.2	26.6	25.9

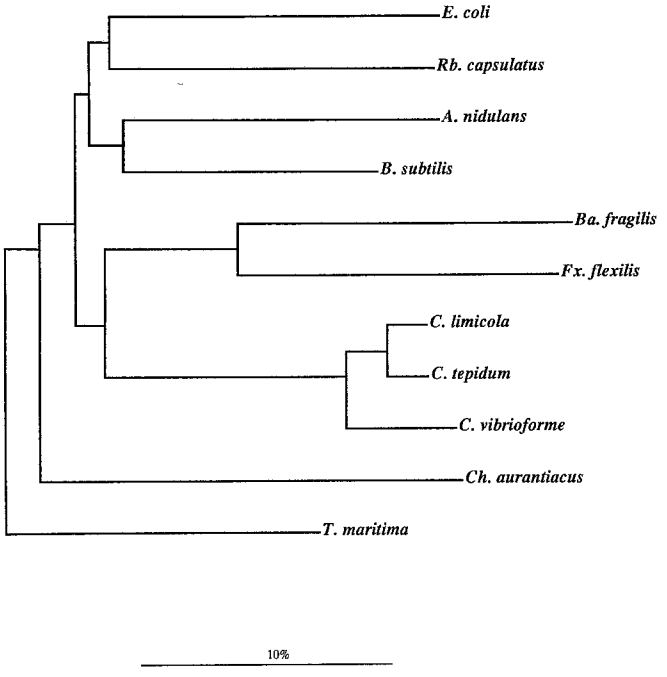

Fig. 5. Phylogenetic position of *Chlorobium tepidum* among other *Chlorobium* species and other phototrophic organisms. Both *C. limicola (vibrioforme)* f. *thiosulfatophilum* and *C. tepidum* are thiosulfate utilizers while *C. vibrioforme* is not. Scale indicates distance on the tree equivalent to 10 nucleotide substitutions per 100 nucleotides in 16S rRNA (see Table 2 for genus names abbreviations). Phylogenetic tree is based upon evolutionary distances given in Table 2. Because it is the deepest branching lineage among the eubacteria, *T. maritima* (Achenbach-Richter et al. 1987) has been used to root the tree

analyses of thermophilic chlorobia (Fig. 5) further supports this.

Chloroflexus is widespread in thermal environments, developing thick laminated mats generally along with cyanobacteria in neutral to alkaline hot springs at temperatures from 40–73° C (Bauld and Brock 1973; Brock 1978; Castenholz and Pierson 1981; Pierson and Castenholz 1974a). Microbial mats in high sulfide hot springs are frequently composed only of anoxygenic phototrophs, usually obligately phototrophic (GCF) strains of *Chloroflexus* (Giovannoni et al. 1987) with or without the purple sulfur bacterium *Chromatium tepidum* (Madigan 1984; 1986). Why thermophilic chlorobia are absent from such springs is not entirely clear, but is probably due to a too alkaline pH and insufficient levels of soluble sulfide. Development of thermophilic chlorobia seems to require a special mix of conditions including sulfide concentrations 5–10 times higher than that found in anoxygenic mats formed by GCF *Chloroflexus* strains and *Chromatium tepidum*, a pH of less than 6.5, and temperatures between 40 and 55° C (Castenholz et al. 1990). Such conditions in thermal springs are rare, however, and it is probably because of these unusual growth requirements that thermophilic chlorobia are relatively limited in geographic distribution (Castenholz 1988; Castenholz et al. 1990). All isolates described herein came from a series of springs in New Zealand which thus far are the only springs known to combine the physical and chemical conditions apparently necessary for development of thermophilic chlorobia (Castenholz et al. 1990). In high sulfide alkaline hot springs, thermophilic chlorobia are probably outcompeted by *Chloroflexus*, whereas in low sulfide springs the thermophilic chlorobia probably cannot coexist with cyanobacteria because oxygen produced by the latter would interfere with their obligately anaerobic metabolism. Only in acidic thermal springs containing high levels of sulfide, where the low pH prevents growth of *Chloroflexus* and the high sulfide and low pH growth of cyanobacteria, can thermophilic chlorobia thrive.

The major properties of the new organism described herein clearly group it within the genus *Chlorobium:* rod-shaped morphology, presence of bacteriochlorophyll *c* and chlorosomes, obligately anaerobic metabolism, nonmotile with absence of gas vesicles, and a 16S ribosomal RNA sequence typical of members of the green sulfur branch of the domain Bacteria (Gibson et al. 1985;

Pfennig and Trüper 1989; Woese et al. 1990a, b). However, a major differentiating property between these hot spring isolates and other species of chlorobia, is thermophily. *Chlorobium* strain TLS is the first green sulfur bacterium shown to be able to grow over 50° C and shows a temperature optimum some 15–20° C above that of known *Chlorobium* species (Pfennig and Trüper 1989). Based on this and other novel properties we propose to describe these thermophilic green bacteria as a new species of the genus *Chlorobium* , *Chlorobium tepidum*. *C. tepidum* can thus be considered a thermophilic member of the family Chlorobiaceae.

Besides its thermophilic phenotype, *C. tepidum* shows other unusual properties. Cultures of the type strain (strain TLS) grow very rapidly, generation times of about 2 h being measured under optimal conditions. By contrast, *Chlorobium* strain 8327 grows with an approximately 6 hour generation time (unpublished results and Kelly 1974). This feature of rapid growth should make *Chlorobium tepidum* an attractive organism for basic studies of photosynthesis in green bacteria; gram quantities of cells can easily be obtained overnight. In addition, *C. tepidum* utilizes thiosulfate as a photosynthetic electron donor and can thus be grown in media containing fairly low levels of sulfide. In this connection it was observed that sulfide alone supported only poor growth of *C. tepidum* and that thiosulfate greatly increased cell yields, even more so than did the addition of an organic compound like acetate. This suggests that *C. tepidum* has a highly developed thiosulfate-oxidizing system but that it may have difficulty oxidizing sulfide to highly-oxidized sulfur species such as sulfate.

The inability of *C. tepidum* to grow appreciably at the expense of sulfide alone is puzzling in light of the high sulfide habitat of this organism, but could actually be a reflection of the unusual growth conditions this organism experiences in situ. In its natural environment *C. tepidum* receives constant levels of geothermal sulfide in the thermal waters that bathe the mats (Castenholz 1988; Castenholz et al. 1990). This is unlike the situation in stratified lakes where phototrophic green bacteria depend on sulfide from biogenic sulfate reduction, a highly intermittant process which is heavily dependent on the concentration of organic matter present (Madigan 1988). And, because green bacteria do not oxidize elemental sulfur to sulfate until sulfide is depleted (Trüper 1978; van Gemerden 1986), it is possible that the high and constant sulfide concentrations present in the environment of *C. tepidum* has over time selected for strains unable to oxidize sulfide fully to sulfate. Thiosulfate, on the other hand, is likely to be oxidized to sulfate by *C. tepidum* because of the precipitous drop in pH which occurs in cultures grown to high densities on excess thiosulfate. Although it is not known whether thiosulfate is present in the natural habitat of *C. tepidum*, the fact that all strains isolated herein were capable of using (and indeed almost required) thiosulfate, suggests that thiosulfate may be a major sulfur species in acidic New Zealand hot springs. Indeed, recent evidence suggests that thiosulfate may be a major component of the sulfur pool of anaerobic habitats, especially marine sediments (Jørgensen 1990).

Like mesophilic chlorobia (Heda and Madigan 1986), thermophilic chlorobia are capable of N_2 fixation. Acetylene reduction occurred at 47–48° C in cell suspensions of *C. tepidum* strain TLS and this is the highest temperature reported for N_2 fixation in any anoxygenic phototroph (Resnick and Madigan 1989). Rates of acetylene reduction by N_2-grown *C. tepidum* cells were quite high, higher even than those of the best nitrogen-fixing nonsulfur purple bacteria (Madigan et al. 1984) and similar to the maximally derepressed nitrogenase activities observed in amino acid-grown mesophilic chlorobia (Heda and Madigan 1986). The nitrogenase system of *C. tepidum* responded to ammonia repression and thus the organism appears to have a typical nitrogenase system in this regard. Although the combined nitrogen content of the habitat of *C. tepidum* is not known, thermal springs typically contain relatively high levels of ammonia (Brock 1978; Castenholz 1969). However, the high density of the *Chlorobium* population in these New Zealand springs may keep NH_3 levels within the mat low. Thus the ability to fix N_2 may be of importance to thermophilic chlorobia in nature.

Lipid analyses of cells of *C. tepidum* indicate a novel lipid composition for thermophilic chlorobia. Cells of *C. tepidum* contain large amounts of wax esters (Shiea et al. 1991), unusual alcohol-fatty acid lipids absent from mesophilic green sulfur bacteria (Knudsen 1982). Wax esters ($C_{28}-C_{38}$) have also been found in *Chloroflexus* (Knudsen 1982; Beyer et al. 1983) but the predominant wax esters of *C. tepidum* are slightly smaller, in the $C_{28}-C_{32}$ range (Shiea et al. 1991). The major fatty acid in extractable lipids of mesophilic chlorobia, n-tetradecanoic acid (C_{14}, Knudsen 1982), is present in only relatively small amounts in cells of *C. tepidum*; the major fatty acid in the latter is palmitic (C_{16}) acid (Shiea et al. 1991). The alcohols present in *C. tepidum* cells are C_{14}, $C_{16:1}$, C_{16}, C_{17}, C_{18} and C_{20} (the latter presumably phytol from bacteriochlorophyll *a*), with C_{16} predominating (Shiea et al. 1991). Although the major bacteriochlorophyll of *C. tepidum* is bchl *c*, lipid analyses (Shiea et al. 1991) have failed to detect isoprenoid alcohols, such as farnesol, in cells of *C. tepidum*. Farnesol is the esterifying alcohol found on bchls *c, d,* and *e* of all other green sulfur bacteria (Gloe et al. 1975). The absence of farnesol in cells of *C. tepidum* suggests that bacteriochlorophyll *c* in this organism is esterified by a longer chain alcohol, perhaps a straight chain C_{16} moiety (Shiea et al. 1991). This would be similar to the finding of a C_{18} alcohol (stearyl alcohol) esterified to bacteriochlorophyll *c* in *Chloroflexus* (Gloe and Risch 1978), a modification undoubtedly evolved to elicit greater heat stability.

Because of the phylogenetic relationships between mesophilic and thermophilic chlorobia (see Fig. 5), it is likely that the intracellular location of pigment complexes in mesophilic chlorobia also holds true for thermophilic forms like *C. tepidum*. Thus bchl *c* and small amounts of bchl *a* (associated with the chlorosome base plate, Wang et al. 1990) would be localized in *C. tepidum* chlorosomes, with the bulk of bchl *a* present in the cytoplasmic membrane. This conclusion is supported by a recent comparative study of chlorosome proteins in mesophilic chlorobia

Table 3. Major properties of *Chlorobium* species

Chlorobium species	Morphology and dimensions width × length (μm)	Pigments: Bchl, major carotenoids and color of cell suspensions	Temp. optimum (°C)	NaCl requirement (%)	DNA base ratio (mol% G + C)	Organic compounds assimilated	Thiosulfate utilization
C. tepidum	Straight or curved rod 0.6–0.8 × 1.3–2.6	Bchl *c* Chlorobactene Dark green	47	0	56.5	Acetate, Pyruvate	+
C. chlorovibrioides	Curved rod, or vibroid 0.3–0.4 × 0.4–0.8	Bchl *d* Chlorobactene Bright green	20–30	2–3	54.0	Acetate, Propionate Formate	–
C. limicola	Straight or curved rod 0.7–1.1 × 0.9–1.5	Bchl *c* or *d* Chlorobactene Green	25–35	0	51.0	Acetate, Glutamate Propionate, Fructose Pyruvate	–
C. limicola f. sp. thiosulfatophilum	Straight or curved rod 0.7–1.1 × 0.9–1.5	Bchl *c* or *d* Chlorobactene Green	25–35	0	58.1	Acetate, Propionate Pyruvate, Glutamate Fructose	+
C. phaeobacteroides	Straight or curved rod 0.6–0.8 × 1.3–2.7	Bchl *e* Isorenieratene Red to brown	20–30	0	49.0	Acetate Fructose	–
C. phaeovibrioides	Curved rod to vibrioid 0.3–0.4 × 0.7–1.4	Bchl *e* Chlorobactene Isorenieratene Light brown to red	20–30	2	53.0	Acetate Propionate	–
C. vibrioforme	Curved rod to vibrioid 0.5–0.7 × 1.0–1.2	Bchl *d* or *c* Chlorobactene Green	25–35	2	53.5	Acetate Propionate	–
C. vibrioforme f. sp. thiosulfatophilum	Curved rod to vibrioid 0.5–0.7 × 1.0–1.2	Bchl *d* or *c* Chlorobactene Green	25–35	2	53.5	Acetate Propionate	+

and *C. tepidum* which suggests that significant homology exists in bchl *a* binding proteins among these organisms (Stolz et al. 1990). However, in light of the major differences in lipid composition between mesophilic and thermophilic chlorobia and *Chloroflexus* (Beyer et al. 1983; Holo et al. 1985; Knudsen et al. 1982; Shiea et al. 1991), it is likely that some aspects of chlorosome structure in the two groups will differ in significant ways. It will be especially interesting to see if the wax esters in the evolutionarily unrelated organisms *Chloroflexus aurantiacus* and *Chlorobium tepidum* have any common functional roles.

Description of the new species, *Chlorobium tepidum*

In view of the differences described herein between thermophilic and mesophilic species of the genus *Chlorobium* (summarized in Table 3), it is proposed to recognize thermophilic green sulfur bacteria as a new species of *Chlorobium, Chlorobium tepidum*:

Chlorobium tepidum sp. nov. tepi.dum. L. neut adj. tepidum, luke warm

Individual cells are rod-shaped and vary in dimensions in different strains from short to long rods. Cells of the type strain measure 0.6–0.8 × 1.3–2.6 μm. Other strains have cells of similar width but can be 10–25 μm in length. Gram negative. Nonmotile. Contain bacteriochlorophyll *c* and small amounts of bacteriochlorophyll *a*. Absorption spectra of intact cells show major peaks at 753 and 460 nm. Major carotenoid probably is chlorobactene. Chlorosomes present. Cells contain C_{28}–C_{32} wax esters.

Obligately phototrophic and anaerobic; sulfide required for growth under all conditions. Electron donors for photoautotrophic growth include sulfide and thiosulfate. Sulfide alone supports only very poor growth, thiosulfate is required to obtain high cell yields. Acetate and pyruvate photoassimilated. Sugars and other organic or fatty acids (including propionate) not utilized. Ammonia, glutamine, and N_2 serve as nitrogen sources. Vitamin B_{12} required. Optimum pH 6.8–7.0. Optimum growth temperature 47–48°C. Maximum temperature 51–52°C; minimum 32°C. NaCl not required for growth. Habitat: High sulfide acidic (pH 4.5–6) thermal springs at temperatures to 55°C.

DNA base ratio: 56.5–58.2 mol% G + C. Type strain TLS (G + C base ratio, 56.5 mol%) isolated from "Travelodge Stream", Sulphur Bay, Rotorua, New Zealand.

16S rRNA sequencing shows strong affinity to green sulfur branch of the eubacterial phylogenetic tree, especially to thiosulfate-utilizing species of *Chlorobium*.

Chlorobium tepidum strain TLS has been deposited in the American Type Culture Collection as ATCC 49652.

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References

Achenbach-Richter LR, Gupta JR, Stetter KO, Woese CR (1987) Were the original eubacteria thermophiles? Syst Appl Microbiol 9:34–39

Bauld J, Brock TD (1973) Ecological studies of *Chloroflexis*, a gliding photosynthetic bacterium. Arch Mikrobiol 92:267–284

Beiji A, Izard D, Gavini F, Leclerc H, Leseine-Delstanche M, Krembel J (1987) A rapid chemical procedure for isolation and purification of chromosomal DNA from Gram-negative bacilli. Anal Biochem 162:18–23

Beyer P, Falk H, Kleinig H (1983) Particulate fractions from *Chloroflexus aurantiacus* and distribution of lipids and polyprenoid forming activities. Arch Microbiol 134:60–63

Biggin MD, Gibson TJ, Hong GF (1983) Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. Proc Natl Acad Sci (USA) 80:3963–3965

Brock TD (1978) Thermophilic microorganisms and life at high temperatures. Springer, Berlin Heidelberg New York

Brosius J, Palmer ML, Kennedy JP, Noller HF (1978) Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. Proc Natl Acad Sci USA 75:4801–4805

Castenholz RW (1969) Thermophilic blue-green algae and the thermal environment. Bacteriol Rev 33:476–504

Castenholz RW (1973) The possible photosynthetic use of sulfide by the filamentous phototrophic bacteria of hot springs. Limnol Oceanogr 18:863–876

Castenholz RW (1977) The effect of sulfide on the blue-green algae of hot springs. II. Yellowstone National Park. Microb Ecol 3:79–105

Castenholz RW (1988) The green sulfur and nonsulfur bacteria of hot springs. In: Olson JM, Ormerod JG, Amesz J, Stackebrandt E, Trüper HG (eds) Green photosynthetic bacteria. Plenum Press, New York, pp 243–255

Castenholz RW, Bauld J, Jørgensen BB (1990) Anoxygenic microbial mats of hot springs: thermophilic *Chlorobium sp.* FEMS Microbiol Ecol 74:325–336

Castenholz RW, Pierson BK (1981) Isolation of members of the family Chloroflexaceae. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes. Springer, Berlin Heidelberg New York, pp 290–298

Cohen-Bazire G, Sistrom WR (1966) The procaryotic photosynthetic apparatus. In: Vernon LP, Seeley GR (eds) The chlorophylls. Academic Press, New York, pp 313–341

De Soete G (1983) A least squares algorithm for fitting additive trees to proximity data. Psychometrika 48:621–626

Gemerden H van (1986) The production of elemental sulfur by green and purple sulfur bacteria. Arch Microbiol 146:52–56

Gibson J, Ludwig W, Stackebrandt E, Woese CR (1985) The phylogeny of the green photosynthetic bacteria: absence of a close relationship between *Chlorobium* and *Chloroflexus*. System Appl Microbiol 6:152–156

Giovannoni SJ, Revsbech NP, Ward DM, Castenholz RW (1987) Obligately phototrophic *Chloroflexus:* primary production in anaerobic hot spring microbial mats. Arch Microbiol 147:80–87

Gloe A, Pfennig N, Brockmann H Jr, Trowitzsch W (1975) A new bacteriochlorophyll from brown-colored Chlorobiaceae. Arch Microbiol 102:103–109

Gloe A, Risch N (1978) Bacteriochlorophyll c_s, a new bacteriochlorophyll from *Chloroflexus aurantiacus*. Arch Microbiol 118:153–156

Green CJ, Stewart GC, Hollis MA, Vold BS, Bott KF (1985) Nucleotide sequence of *Bacillus subtilis* ribosomal RNA operon, *rrnB*. Gene 37:261–266

Heda GD, Madigan MT (1986) Aspects of nitrogen fixation in *Chlorobium*. Arch Microbiol 143:330–336

Holo H, Brock-Due M, Ormerod JG (1985) Glycolipids and the structure of chlorosomes in green bacteria. Arch Microbiol 143:94–99

Jørgensen BB (1990) A thiosulfate shunt in the sulfur cycle of marine sediments. Science 249:152–154

Jukes TH, Cantor R (1969) Evolution of protein molecules. In: Munro HN (ed) Mammalian protein metabolism. Academic Press, New York, pp 21–132

Kelly DP (1974) Growth and metabolism of the obligate photolithotroph *Chlorobium thiosulfatophilum* in the presence of added organic nutrients. Arch Microbiol 100:163–178

Knudsen E, Jantzen E, Bryn K, Ormerod JG, Sirevåg R (1982) Quantitative and structural characteristics of lipids in *Chlorobium* and *Chloroflexus*. Arch Microbiol 132:149–154

Lane DJ, Pace B, Olsen GJ, Stahl DA, Sogin ML, Pace NR (1985) Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc Natl Acad Sci USA 82:6955–6959

Madigan MT (1984) A novel photosynthetic purple bacterium isolated from a Yellowstone hot spring. Science 225:313–315

Madigan MT (1986) *Chromatium tepidum* sp. nov., a thermophilic photosynthetic bacterium of the family *Chromatiaceae*. Int J Syst Bacteriol 36:222–227

Madigan MT (1988) Microbiology, physiology and ecology of phototrophic bacteria. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. Wiley, New York, pp 39–111

Madigan MT, Brock TD (1975) Photosynthetic sulfide oxidation by *Chloroflexus aurantiacus*, a filamentous, photosynthetic, gliding bacterium. J Bacteriol 122:782–784

Madigan MT, Cox SS, Stegeman RA (1984) Nitrogen fixation and nitrogenase activities in members of the family *Rhodospirillaceae*. J Bacteriol 157:73–78

Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J Mol Biol 3:208–218

Olson JM (1980) Chlorophyll organization in green photosynthetic bacteria. Biochim Biophys Acta 594:33–51

Pfennig N, Trüper HG (1989) Anoxygenic phototrophic bacteria. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 3. Williams & Wilkins, Baltimore, pp 1635–1709

Pierson BK, Castenholz RW (1974a) A phototrophic gliding filamentous bacterium of hot springs, *Chloroflexus aurantiacus*, gen. and sp. nov. Arch Microbiol 100:5–24

Pierson BK, Castenholz RW (1974b) Studies of pigments and growth in *Chloroflexus aurantiacus*, a phototrophic filamentous bacterium. Arch Microbiol 100:283–305

Pierson BK, Castenholz RW (1991) The anoxygenic phototrophic bacteria (Family Chloroflexaceae). In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes, 2nd ed., Springer, Berlin Heidelberg New York (in press)

Pierson BK, Giovannoni SJ, Castenholz RW (1984) Physiological ecology of a gliding bacterium containing bacteriochlorophyll *a*. Appl Environ Microbiol 47:576–584

Pierson BK, Giovannoni SJ, Stahl DA, Castenholz RW (1985) *Heliothrix oregonensis*, gen. nov., sp. nov., a phototrophic filamentous gliding bacterium containing bacteriochlorophyll *a*. Arch Microbiol 142:164–167

Resnick S, Madigan MT (1989) Isolation and characterization of a mildly thermophilic nonsulfur purple bacterium containing bacteriochlorophyll *b*. FEMS Microbiol Lett 65:165–170

Ryter A, Kellenberger E, Birch-Andersen A, Maaløe O (1958) Etude au microscope électronique de plasma contenant de l'acide desoxyribonucléique I. Les nucleoides des bactéries en croissance active. Z Naturforsch 136:597–605

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467

Schmidt K (1980) A comparative study on the composition of chlorosomes (Chlorobium vesicles) and cytoplasmic membranes from *Chloroflexus aurantiacus* strain OK-70-fl and *Chlorobium limicola* f. *thiosulfatophilum* strain 6230. Arch Microbiol 124: 21–31

Shiea J, Brassell SC, Ward DM (1991) Comparative analysis of extractable lipids in hot spring microbial mats and their component photosynthetic bacteria. Org Geochem 17:309–319

Stanier RY, Smith JHC (1960) The chlorophylls of green bacteria. Biochim Biophys Acta 41:478–484

Stolz JF, Fuller RC, Redlinger TE (1990) Pigment-protein diversity in chlorosomes of green phototrophic bacteria. Arch Microbiol 154:422–427

Trüper HG (1978) Sulfur metabolism. In: Clayton RK, Sistrom WR (eds) The photosynthetic bacteria. Plenum Press, New York, pp 269–274

Wang J, Brune DC, Blakenship RE (1990) Effects of oxidants and reductants on the efficiency of excitation transfer in green photosynthetic bacteria. Biochim Biophys Acta 1015:457–463

Ward DM, Weller R, Shiea J, Castenholz RW, Cohen Y (1989) Hot spring microbial mats: anoxygenic and oxygenic mats of possible evolutionary significance. In: Cohen Y, Rosenberg E (eds) Microbial mats – physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, DC, pp 3–15

Weisburg WG, Tully JG, Rose DL, Petzel JP, Oyaizu H, Yang D, Mandelco L, Sechrest J, Lawrence TG, van Etten J, Maniloff J, Woese CR (1989) A phylogenetic analysis of the mycoplasmas: basis for their classification. J Bacteriol 171:6455–6467

Woese CR, Stackebrandt E, Macke TJ, Fox GE (1985) A phylogenetic definition of the major eubacterial taxa. Syst Appl Microbiol 6:143–151

Woese CR, Kandler O, Wheelis ML (1990a) Toward a natural system of organisms: Proposals for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 87:4576–4579

Woese CR, Mandelco L, Yang D, Gherna R, Madigan MT (1990b) The case for relationship of the flavobacteria and their relatives to the green sulfur bacteria. Syst Appl Microbiol 13:258–262