

Phylogenetic taxonomy of the family *Chlorobiaceae* on the basis of 16S rRNA and *fmo* (Fenna–Matthews–Olson protein) gene sequences

Johannes F. Imhoff

Correspondence
Johannes F. Imhoff
jimhoff@ifm.uni-kiel.de

Institut für Meereskunde an der Universität Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

A new taxonomy of the green sulfur bacteria is proposed, based on phylogenetic relationships determined using the sequences of the independent 16S rRNA and *fmo* (Fenna–Matthews–Olson protein) genes, and supported by the DNA G+C content and sequence signatures. Comparison of the traditional classification system for these bacteria with their phylogenetic relationship yielded a confusing picture, because properties used for classification (such as cell morphology, photosynthetic pigments and substrate utilization) do not concur with their phylogeny. Using the genetic information available, strains and species assigned to the genera *Chlorobium*, *Pelodictyon* and *Prosthecochloris* are considered, and the following changes are proposed. *Pelodictyon luteolum* is transferred to the genus *Chlorobium* as *Chlorobium luteolum* comb. nov. *Pelodictyon clathratiforme* and *Pelodictyon phaeoclathratiforme* are transferred to the genus *Chlorobium* and combined into one species, *Chlorobium clathratiforme* comb. nov. The name *Pelodictyon* will become a synonym of *Chlorobium*. Strains known as *Chlorobium limicola* subsp. *thiosulfatophilum* that have a low DNA G+C content (52–52.5 mol%) are treated as strains of *Chlorobium limicola*; those with a high DNA G+C content (58.1 mol%) are transferred to *Chlorobaculum* gen. nov., as *Chlorobaculum thiosulfatophilum* sp. nov. *Chlorobium tepidum* is transferred to *Chlorobaculum tepidum* comb. nov., and defined as the type species of the genus *Chlorobaculum*. Strains assigned to *Chlorobium phaeobacteroides*, but phylogenetically distant from the type strain of this species, are assigned to *Chlorobium limicola* and to *Chlorobaculum limnaeum* sp. nov. Strains known as *Chlorobium vibrioforme* subsp. *thiosulfatophilum* are transferred to *Chlorobaculum parvum* sp. nov. *Chlorobium chlorovibrioides* is transferred to '*Chlorobaculum chlorovibrioides*' comb. nov. The type strain of *Chlorobium vibrioforme* is phylogenetically related to *Prosthecochloris*, and is therefore transferred to this genus as *Prosthecochloris vibrioformis* comb. nov. Consequently, the name *Chlorobium vibrioforme* will become a synonym of *Prosthecochloris vibrioformis*, and other strains that were assigned to this species are now considered to belong to *Chlorobium luteolum*, *Chlorobium phaeovibrioides* and '*Chlorobaculum chlorovibrioides*', according to their phylogenetic relatedness.

Introduction

The green sulfur bacteria, represented by the family *Chlorobiaceae*, form a branch of bacteria that is phylogenetically distinct from other main phylogenetic lines, and is therefore treated as a separate phylum in *Bergey's Manual of Systematic Bacteriology* (Overmann, 2001). Traditionally, the taxonomic classification of these bacteria is based on morphological and easily recognizable phenotypic properties (Pfennig, 1989; Pfennig & Overmann, 2001a, b); such characteristic properties include cell morphology, pigment

composition and absorption spectra, and metabolic properties. In particular, (i) the formation of gas vesicles has been used to distinguish between genera; (ii) brown-coloured forms have been distinguished as species from their green-coloured counterparts, and are distinct in their bacteriochlorophyll and carotenoid compositions; and (iii) subspecies were recognized on the basis of utilization of thiosulfate as a photosynthetic electron donor. Although these properties are easily recognizable and have allowed a clear phenotypic differentiation, they are not in accord with the phylogenetic relationship of these bacteria (Figueras *et al.*, 1997; Overmann & Tuschak, 1997). Therefore, in a systematic taxonomy of green sulfur bacteria based on phylogenetic relationships, they have only very limited relevance.

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Abbreviations: BChl, bacteriochlorophyll; *fmo*, Fenna–Matthews–Olson.

Recently, phylogenetic relationships of green sulfur bacteria were established by using 16S rRNA and *fmo* (Fenna–Matthews–Olson protein, FMO protein) gene sequences, including important signatures of amino acid and nucleotide sequences (Alexander *et al.*, 2002). The congruent phylogenetic relationships found with two independent gene sequences provide a solid basis for the phylogeny of these bacteria, and for a phylogeny-based taxonomy. A preliminary discussion of taxonomic consequences for the green sulfur bacteria and some proposals were presented previously (Imhoff, 1999; Alexander *et al.*, 2002). Based on these data, some basic taxonomic changes are proposed to establish a phylogenetic system of classification. Genera and species of the green sulfur bacteria are defined, and most available strains are reassigned.

Organisms

The following taxonomic proposals consider strains and species assigned to the genera *Chlorobium*, *Prosthecochloris* and *Pelodictyon*. Altogether, 15 species and subspecies of these three genera are known (Gorlenko, 2001; Heising *et al.*, 1999; Pfennig & Overmann, 2001a, b), and both 16S rRNA and *fmo* gene sequence information is available for 10 of the type strains. Additional 16S rDNA sequences of a newly described species ('*Chlorobium ferrooxidans*') and two species where type strains have been lost (*Pelodictyon clathratiforme* and *Chlorobium chlorovibrioides*) are also available (Table 1). In addition, *fmo* gene sequences have been determined for 17 non-type strains (Alexander *et al.*, 2002), and a larger number of 16S rDNA sequences is available (Figueras *et al.*, 1997; Overmann & Tuschak, 1997; Alexander *et al.*, 2002).

Type strain material from *Pelodictyon phaeum* and *Prosthecochloris phaeoasteroidea* did not exist, and neither 16S rRNA nor *fmo* gene sequence information was available for either species; therefore, they are not considered in the following. Because of the lack of type strains, *Ancalochloris perfilievii* and '*Clathrochloris sulfurica*' (for which no species description has been published, but a 16S rDNA sequence is available; Witt *et al.*, 1989) are not included in the following considerations.

Chloroherpeton thalassium forms a clearly separate phylogenetic line from the species and genera discussed here. It represents a well-established genus and species, and does not need to be included in the following considerations.

Phylogenetic results

The phylogenetic studies revealed almost-identical grouping in trees constructed from 16S rDNA and *fmoA* sequences, and suggest a largely congruent evolution of FMO and 16S rDNA (Alexander *et al.*, 2002). This is quite remarkable, and strongly supports the 16S rDNA-based phylogeny of these bacteria. The assignment of strains into major phylogenetic groups is further supported by characteristic signatures in the amino acid sequence of the FMO protein (Alexander *et al.*, 2002). Their phylogenetic relationship

and separation into groups is depicted in Fig. 1, which shows the 16S rDNA-based relationships between the strains compared to those based on *fmo* sequences, and in Table 1.

The phylogenetic grouping of the green sulfur bacteria is not in accord with their current classification, or with the current assignment of species to genera and of strains to species. Three examples demonstrate this: (i) species of the genus *Chlorobium* appear in all major groups, and those of the genus *Pelodictyon* in two of them (Table 1); (ii) strains of several species, including *Chlorobium limicola*, *Chlorobium phaeobacteroides*, *Chlorobium vibrioforme* and *Chlorobium phaeovibrioides*, appear in at least two of the major groups (Table 1); (iii) *Chlorobium limicola* subsp. *thiosulfatophilum* and *Chlorobium vibrioforme* subsp. *thiosulfatophilum*, as defined by their type strains, are different from their reference species at the genus level (Table 1).

From analysis of all available information, it becomes obvious that besides 16S rRNA and other gene sequences, the DNA G + C content is relevant for species recognition. It is difficult at present to find phenotypic properties that can be determined easily, and that are in agreement with the genetic relationship between the green sulfur bacteria. Properties that are significant for taxonomic classification include the requirement for salt, and the composition of lipids and fatty acids may also prove to be useful (Imhoff, 1988; Imhoff & Bias-Imhoff, 1995). However, properties that have been used so far for classification (pigment composition, presence of gas vesicles, thiosulfate utilization and the characteristic formation of cell aggregates) are apparently of very limited significance in a phylogenetic system of the green sulfur bacteria.

Four major groups of species of green sulfur bacteria were recognized by Alexander *et al.* (2002), and were proposed as a basis for the definition of genera. Only two of these groups currently have recognized type species: *Chlorobium limicola* and *Pelodictyon clathratiforme* (group 3; Alexander *et al.*, 2002) and *Prosthecochloris aestuarii* (group 1; Alexander *et al.*, 2002). Group 4 is represented by a number of strains and species presently assigned to the genus *Chlorobium*, although phylogenetically distant from the type species *Chlorobium limicola* and the group of strains that clusters with this species. Consequently, the bacteria of group 4 need to be assigned to a novel genus, for which the name *Chlorobaculum* gen. nov. is proposed.

Although the consideration of sequence signatures of the FMO protein made the separation of groups 2 and 3 of Alexander *et al.* (2002) both possible and plausible, these two groups are quite closely related and the clear separation of the strains poses problems. According to 16S rRNA gene sequences, the type strains of *Chlorobium phaeovibrioides*, *Chlorobium vibrioforme* subsp. *thiosulfatophilum* and *Pelodictyon luteolum* form the coherent cluster of group 2. In particular, subgroup 3a (which includes *Chlorobium phaeobacteroides* and *Pelodictyon phaeoclathratiforme*) has an intermediate position between the two groups, and is

Table 1. Names and some properties of species and strains of the genera *Chlorobium*, *Prosthecochloris* and *Chlorobaculum*

Genus and species name	Previous name	Strain no.	Thiosulfate used	Cell size (µm)	Salt required	Vitamins	Major BChl	DNA G+C content (mol%)	Gas vesicles	Carotenoid*	Major fatty acids (% of total)		
											C _{14:0}	C _{16:0}	C _{16:1}
<i>Chlorobium</i>													
Group 3b†													
<i>Chlorobium limicola</i>	<i>Chlorobium limicola</i>	DSM 245 ^T		0.7–1.1	No	–	<i>c</i>	51.0	–	clb	13	17	57
<i>Chlorobium limicola</i>	<i>Chlorobium limicola</i>	DSM 246			No	B ₁₂	<i>c</i>	52.0	–	clb			
<i>Chlorobium limicola</i>	<i>Chlorobium limicola</i> subsp. <i>thiosulfatophilum</i>	1630	+		No	B ₁₂	<i>c</i>	52.5	–				
<i>Chlorobium limicola</i>	<i>Chlorobium limicola</i> subsp. <i>thiosulfatophilum</i>	9330	+		No	–	<i>c</i>	52.0	–				
<i>Chlorobium limicola</i>	<i>Chlorobium phaeobacteroides</i>	DSM 1855	–		No	B ₁₂	<i>e</i>		–	iso			
	<i>Chlorobium limicola</i>	DSM 247	–		No	B ₁₂	<i>c</i>	51.5	–				
	<i>Chlorobium limicola</i>	DSM 248	–		No	B ₁₂	<i>c</i>	51.5	–				
	<i>Chlorobium limicola</i> subsp. <i>thiosulfatophilum</i>	DSM 257	+		No	–	<i>c</i>	52.5					
Group 3a†													
<i>Chlorobium phaeobacteroides</i>	<i>Chlorobium phaeobacteroides</i>	DSM 266 ^T	–	0.6–0.8	No	B ₁₂	<i>e</i>	49.0	–	iso	16	15	64
	<i>Chlorobium phaeobacteroides</i>	DSM 267	–		No	B ₁₂	<i>e</i>	50.0		iso			
<i>Chlorobium clathratiforme</i>	<i>Pelodictyon phaeoclathratiforme</i>	DSM 5477 ^T	+	0.8–1.1	No	B ₁₂	<i>e</i>	47.9	+	iso			
<i>Chlorobium clathratiforme</i>	<i>Pelodictyon clathratiforme</i>	PG	–	0.7–1.2	No			(48.5)	+	clb			
' <i>Chlorobium ferrooxidans</i> '	' <i>Chlorobium ferrooxidans</i> '	DSM 13031 ^T		0.5	No		<i>c</i>		–	clb			
Group 2a†													
<i>Chlorobium luteolum</i>	<i>Pelodictyon luteolum</i>	DSM 273 ^T	–	0.6–0.9	> 1%	B ₁₂	<i>c</i>	58.1	+	clb	14	21	47
<i>Chlorobium luteolum</i>	<i>Chlorobium vibrioforme</i>	DSM 262	–	0.5–0.7	> 1%	B ₁₂	<i>d</i>	57.1	–	clb			
Group 2b†													
<i>Chlorobium phaeovibrioides</i>	<i>Chlorobium phaeovibrioides</i>	DSM 269 ^T	–	0.3–0.4	> 1%	B ₁₂	<i>e</i>	53.0	–	iso	10	29	51
<i>Chlorobium phaeovibrioides</i>	<i>Chlorobium vibrioforme</i> subsp. <i>thiosulfatophilum</i>	DSM 265	–	0.5–0.7	> 1%	B ₁₂	<i>d,c</i>	53.5	–		12	23	52
<i>Chlorobium phaeovibrioides</i>	<i>Chlorobium vibrioforme</i>	DSM 261	–		> 1%	B ₁₂	<i>d</i>	52.0	–				
<i>Chlorobium phaeovibrioides</i>	<i>Chlorobium phaeovibrioides</i>	DSM 270	–		> 1%	B ₁₂	<i>e</i>	52.0	–				

Table 1. cont.

Genus and species name	Previous name	Strain no.	Thiosulfate used	Cell size (μm)	Salt required	Vitamins	Major BChl	DNA G+C content (mol%)	Gas vesicles	Carotenoid*	Major fatty acids (% of total)		
											C _{14:0}	C _{16:0}	C _{16:1}
<i>Prosthecochloris</i>													
Group 1†													
<i>Prosthecochloris aestuarii</i>	<i>Prosthecochloris aestuarii</i>	DSM 271 ^T	–	0.5–0.7	2–5 %	B ₁₂	<i>c</i>	52–56.1	–	clb			
<i>Prosthecochloris</i> sp.	<i>Prosthecochloris aestuarii</i>	2K	–		Yes				–				
<i>Prosthecochloris vibrioformis</i>	<i>Chlorobium vibrioforme</i>	DSM 260 ^T	–	0.5–0.7	> 1 %	B ₁₂	<i>d,c</i>	53.5	–	clb			
<i>Prosthecochloris vibrioformis</i>	<i>Chlorobium phaeovibrioides</i>	DSM 1678	–		> 1 %		<i>e</i>		–				
<i>Prosthecochloris vibrioformis</i>	<i>Chlorobium vibrioforme</i>	CHP 3402							–				
<i>Chlorobaculum</i>													
Group 4b†													
<i>Chlorobaculum tepidum</i>	<i>Chlorobium tepidum</i>	ATCC 49652 ^T	+	0.6–0.8	No		<i>c</i>	56.5	–	clb			
<i>Chlorobaculum limnaeum</i>	<i>Chlorobium phaeobacteroides</i>	DSM 1677 ^T	–		No	B ₁₂	<i>e</i>		–				
<i>Chlorobaculum limnaeum</i>	<i>Chlorobium phaeobacteroides</i>	1549	–		No				–				
	<i>Chlorobium limicola</i>	UdG 6040		0.8–1.0			<i>c</i>		–	clb			
	<i>Chlorobium limicola</i>	UdG 6042		0.8–1.0			<i>c</i>		–	clb			
	<i>Chlorobium limicola</i>	UdG 6045		0.7–1.0			<i>c</i>		–	clb			
	<i>Chlorobium limicola</i>	UdG 6038		0.7–1.0			<i>c</i>		–	clb			
<i>Chlorobaculum thiosulfatophilum</i>	<i>Chlorobium limicola</i> subsp. <i>thiosulfatophilum</i>	DSM 249 ^T	+	0.7–1.0	No	–	<i>c</i>	58.1	–	clb	21	10	43
<i>Chlorobaculum thiosulfatophilum</i>	<i>Chlorobium limicola</i> subsp. <i>thiosulfatophilum</i>	1430	+		No	–	<i>c</i>	58.1	–				
Group 4a†													
<i>Chlorobaculum parvum</i>	<i>Chlorobium vibrioforme</i> subsp. <i>thiosulfatophilum</i>	DSM 263 ^T	+	0.7–1.1	> 1 %		<i>d</i>	56.6	–	clb	24	23	43
<i>Chlorobaculum parvum</i>	<i>Chlorobium vibrioforme</i> subsp. <i>thiosulfatophilum</i>	NCIB 8346	+		> 1 %		<i>d</i>	56.1	–				
' <i>Chlorobaculum chlorovibrioides</i> '	<i>Chlorobium chlorovibrioides</i>	UdG 6026	–	0.3–0.4†	2–3 %	B ₁₂	<i>c</i>	54.0	–	clb			
' <i>Chlorobaculum chlorovibrioides</i> '	<i>Chlorobium vibrioforme</i>	UdG 6043		0.7–0.8	5 %		<i>c</i>		–	clb			

*clb, Chlorobactene; iso, isorenieratene.

†According to Alexander *et al.* (2002).

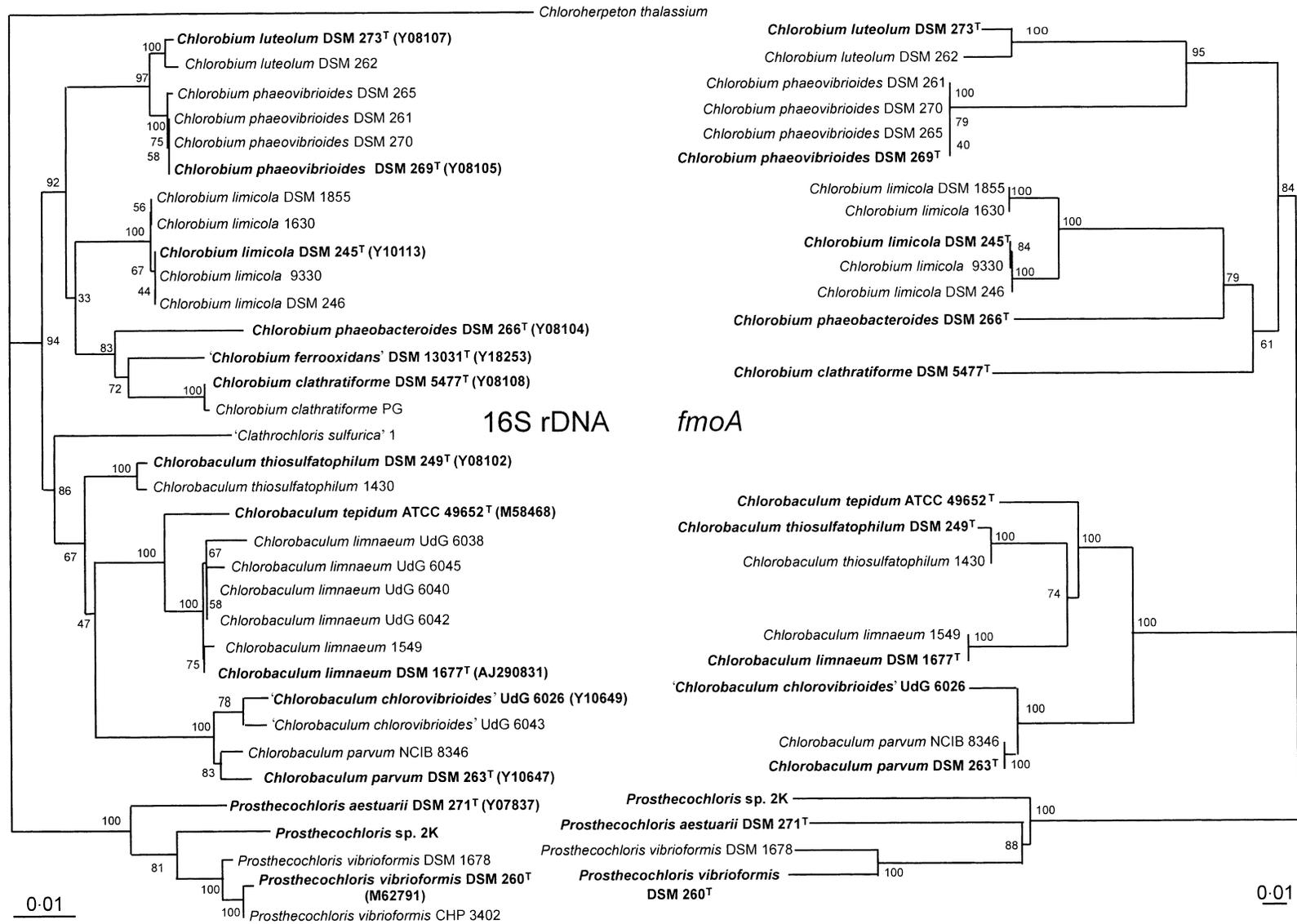


Fig. 1. Phylogenetic trees of 16S rDNA sequences (left) and *fmoA* gene sequences (right) of green sulfur bacteria. Sequence analyses and tree construction are essentially as described by Alexander *et al.* (2002): the sequences were aligned using CLUSTALW or ARB, calculations were made according to the maximum-likelihood method, by using distance matrices for least-square analysis and by parsimony. Because the overall branching patterns were highly similar using different methods of alignment and calculation, for the sake of clarity, trees based on CLUSTALW alignment and distance methods are presented here. Bootstrap analyses with 100 resamplings were performed to obtain confidence estimates for phylogenetic tree topologies. The 16S rDNA tree topology is based on approximately 1220 nt, obtained with the primer pair F-99-GSB/R-1369 (Alexander *et al.*, 2002). Accession numbers of all sequences were published previously (Alexander *et al.*, 2002).

closely related to *Pelodictyon luteolum* on the basis of *fmo* gene sequences. Even if phenotypic information (such as cell morphology, salt requirement and DNA G+C content) were available to distinguish between groups 2 and 3, it is proposed that the species of both groups should be classified in a single genus. According to the presence of the type strain of the type species, *Chlorobium limicola* DSM 245^T, in group 3 and the priority of *Chlorobium* over *Pelodictyon*, *Chlorobium* will be the genus name of this group. The 16S rDNA sequence similarities of more than 95% between strains of this genus, as defined by phylogenetic groups 2 and 3, are in line with current practice, by which separation into genera occurs below 95% similarity in most cases. Consequently, *Chlorobium* will continue to be a heterogeneous genus, in which different subgroups can be distinguished on the basis of properties such as cell form and size, DNA G+C content and salt response, but also on the basis of genetic sequence information. Most of the currently used species names will be maintained, because the majority of species and strains of these two groups were previously included in the genus *Chlorobium*.

Descriptions of genera and species

According to their phylogenetic relationship and the proposed phylogenetic definition of the genera, several species descriptions need to be emended, and a large number of strains need to be reassigned to different species. Properties of the species and genera are given in Table 2. The basic information for strain and species assignment and some properties are shown in Table 1. Proposed new and old names are listed in this table and demonstrate the problematic situation of the current state of the taxonomy. Incorrect species names assigned to a great number of strains of green sulfur bacteria create a highly confusing situation, and limit the transparency of comparative scientific work on these bacteria (particularly if species names but not strain numbers are given, as in many publications).

Because of the priority of types in taxonomy, the defined types set the frame for the following considerations. *Chlorobium* is the type genus of the *Chlorobiaceae* (Trüper, 1976). *Chlorobium limicola*, *Pelodictyon clathratiforme* and *Prosthecochloris aestuarii* are the type species of the genera that are considered for the present discussion (Gorlenko, 2001; Pfennig & Overmann, 2001a, b). Therefore, the type strains of *Chlorobium limicola* (DSM 245^T) and of *Prosthecochloris aestuarii* (DSM 271^T) form the basis of the genera *Chlorobium* and *Prosthecochloris*, which are at a phylogenetic distance that clearly allows their assignment to different genera. *Pelodictyon clathratiforme* is phylogenetically closely related to *Chlorobium limicola*, and must therefore be regarded as a *Chlorobium* species. Because *Pelodictyon clathratiforme* is the type species of this genus, according to Rule 37a of the *Bacteriological Code* (1990 Revision) (Lapage *et al.*, 1992), the consequence of this transfer is that the other *Pelodictyon* species must also be assigned to another genus (see below).

Table 2. Properties of genera and species of the green sulfur bacteria

Genus and species name	Thiosulfate used	Cell size (µm)	Salt required	Vitamins	Major BChl	DNA G+C content	Gas vesicles	Carotenoid*	Major fatty acids (% of total)		
									14:0	16:0	16:1
<i>Chlorobium</i>	+/-	0.3-1.2	+/-	B ₁₂	c,d,e	48-58.1	+/-	clb, iso	14-16	15-29	47-64
<i>Chlorobium limicola</i>	+/-	0.7-1.1	No	B ₁₂	c (or e)	51.0-52.5	-	clb, iso	13	17	57
<i>Chlorobium phaeobacteroides</i>	-	0.6-0.8	No	B ₁₂	e	49.0-50.0	-	iso	16	15	64
<i>Chlorobium clathratiforme</i>	+/-	0.7-1.2	No	B ₁₂	c or e	47.9-49.0	+	clb, iso			
' <i>Chlorobium ferrooxidans</i> '		0.5	No		c			clb			
<i>Chlorobium luteolum</i>	-	0.6-0.9	>1%	B ₁₂	c,d	57.1-58.1	+/-	clb	14	21	47
<i>Chlorobium phaeovibrioides</i>	+/-	0.3-0.7	>1%	B ₁₂	c,d,e	52.0-53.5	-	clb, iso	10-12	23-29	51-52
<i>Prosthecochloris</i>	-	0.5-0.7	>1-5%	B ₁₂	c,d,e	52-56.1	-	clb, iso			
<i>Prosthecochloris aestuarii</i>	-	0.5-0.7	2-5%	B ₁₂	c	52-56.1	-	clb			
<i>Prosthecochloris vibrioformis</i>	-	0.5-0.7	>1%	B ₁₂	c,d,e	53.5	+/-	clb, iso			
<i>Chlorobaculum</i>	+/-	0.3-1.1	0-3%	B ₁₂	c,d,e	54-58.1	-	clb, iso	21-24	10-23	43
<i>Chlorobaculum tepidum</i>	+	0.6-0.8	No	B ₁₂	c	56.5	-	clb			
<i>Chlorobaculum limnaeum</i>	+	0.6-0.8	No	B ₁₂	e		-	iso			
<i>Chlorobaculum thiosulfatiphilum</i>	+	0.7-1.1	No	B ₁₂	c	58.1	-	clb	21	10	43
<i>Chlorobaculum parvum</i>	+	0.7-1.1	>1%		d	56.1-56.6	-	clb	24	23	42.8
' <i>Chlorobaculum chlorovibrioides</i> '	-	0.3-0.4	2-3%	B ₁₂	d	54.0	-	clb			

*clb, Chlorobactene; iso, isorenieratene.

Emended description of the genus *Chlorobium*

The genus *Chlorobium* comprises green sulfur bacteria with vibrioid or rod-shaped cells that occur singly or in cell aggregates. Some species and strains may contain gas vesicles. Cell size ranges from 0.3 to 1.2 µm in width. As photosynthetic pigments, either bacteriochlorophyll (BChl) *c* and *d* or BChl *e* is present. The former are accompanied by carotenoids of the chlorobactene series, and the latter by those of the isorenieratene series. Rod-shaped cells have a cell width of 0.6–1.2 µm, DNA G+C content of 47.9–52.5 mol% and do not require salt; vibrioid species have a cell width of 0.3–0.9 µm, DNA G+C content of 52–58.1 mol% and require low amounts of salt. Most strains require vitamin B₁₂ as a growth factor. The fatty acid composition is characterized by approximately 10–16% C_{14:0} and 57–64% C_{16:1}. The genus comprises strains and species that belong phylogenetically to groups 2 and 3 of the green sulfur bacteria, according to Alexander *et al.* (2002). Photolithoautotrophic growth occurs under anoxic conditions in the light, with reduced sulfur compounds such as sulfide and elemental sulfur as electron donors. Thiosulfate and hydrogen may be used. During oxidation of sulfide, elemental sulfur is transiently deposited outside cells. Final oxidation product is sulfate. In the presence of sulfide and bicarbonate, simple organic substrates are photo-assimilated. Obligately phototrophic and strictly anaerobic. The DNA G+C content is 47.9–58.1 mol% (Bd).

The type species is *Chlorobium limicola* Nadson 1906, 190.

Emended description of *Chlorobium limicola*

The species description is as given by Pfennig & Overmann (2001a), with the following modifications. Cells are rod-shaped, 0.7–1.1 µm wide and non-motile. Photosynthetic pigments are BChl *c* with chlorobactene as the major carotenoid, or in some strains, BChl *e* with isorenieratene. Photoautotrophic growth occurs with sulfide and sulfur as photosynthetic electron donors; molecular hydrogen and thiosulfate may be used. In the presence of sulfide and bicarbonate, some simple organic compounds are photo-assimilated. Freshwater bacteria without a requirement for sodium chloride. The DNA G+C content is 51–52.5 mol%.

The type strain is DSM 245^T. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is Y10113.

Comments. This species is a representative of group 3, according to Alexander *et al.* (2002). In addition to the type strain DSM 245^T, strains clearly assigned to this species are 1630, 9330, DSM 246 and DSM 1855. These include two strains that were formerly known as *Chlorobium limicola* subsp. *thiosulfatophilum* (strains 1630 and 9330) and one strain formerly assigned to *Chlorobium phaeobacteroides* (DSM 1855). Because of the conflicting situation with other strains known as *Chlorobium limicola* subsp. *thiosulfatophilum* that are no longer considered

to belong to the genus *Chlorobium* (see below), it is proposed to abandon the use of subspecies names for thiosulfate-utilizing strains of this and other species of the green sulfur bacteria. Thiosulfate utilization is considered to be a property of some of the strains of *Chlorobium limicola* (strains 1630, 9330 and DSM 257). Although the DNA G+C content and some other properties of strains DSM 247, DSM 248 and DSM 257 fit with the properties of *Chlorobium limicola*, sequence data are lacking and therefore their assignment to *Chlorobium limicola* is likely but not secure. Excluded from this species are all strains that have a DNA G+C content of >54 mol% and belong to group 4 (Table 1). Strains UdG 6051, UdG 6046 and UdG 6047 (known as *Chlorobium phaeobacteroides*; Figueras *et al.*, 1997) are genetically related to *Chlorobium limicola*, but further studies are necessary to determine their relationship to *Chlorobium limicola*.

Emended description of *Chlorobium phaeobacteroides*

The species description is as given by Pfennig & Overmann (2001a). This bacterium is a freshwater species without a salt requirement and with a DNA G+C content of 49–50 mol%.

The type strain is DSM 266^T. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is Y08104.

Comments. This species is a representative of group 3, according to Alexander *et al.* (2002). The type strain of *Chlorobium phaeobacteroides*, DSM 266^T, forms a distinct phylogenetic lineage and is the only strain representing this species. The lack of sequence information does not allow the secure assignment of strain DSM 267 to this species, although its DNA G+C content of 50.0 mol% is close to that of the type strain (49.0 mol%). Other strains known as *Chlorobium phaeobacteroides* must be excluded from this species, and are assigned to *Chlorobium limicola* (DSM 1855) and to *Chlorobaculum limnaeum* (strains 1549 and DSM 1677, see below).

Emended description of '*Chlorobium ferrooxidans*'

The species description is as given by Heising *et al.* (1999). The type strain is DSM 13031^T. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is Y18253.

Comments. This species is a representative of group 3, according to Alexander *et al.* (2002). It is related to, but distinct from, *Chlorobium phaeobacteroides*.

Description of *Chlorobium clathratiforme* comb. nov.

Basonym: *Pelodictyon clathratiforme* (Szafer 1911) Lauterborn 1913 (Approved Lists 1980).

The species description is as given by Pfennig & Overmann (2001b) for *Pelodictyon clathratiforme* and *Pelodictyon phaeoclathratiforme*. Cells are rod-shaped, non-motile, 0.7–1.2 µm wide, produce gas vesicles and characteristically form three-dimensional nets. Colour of cell suspensions is green or brown, and photosynthetic pigments are BChl *c* with chlorobactene as the major carotenoid in green strains and BChl *e* with isorenieratene as the major carotenoid in brown strains. Photoautotrophic growth occurs with sulfide and sulfur as photosynthetic electron donors; thiosulfate may be used. In the presence of sulfide and bicarbonate, some simple organic compounds are photoassimilated. Vitamin B₁₂ is required for growth. Freshwater bacteria without a requirement for NaCl. The DNA G+C content is 48–49 mol%.

The type strain is DSM 5477^T = BU1^T. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is Y08108.

Comments. This species is a representative of group 3, according to Alexander *et al.* (2002). The 16S rDNA sequences of *Pelodictyon clathratiforme* PG and *Pelodictyon phaeoclathratiforme* DSM 5477^T are almost identical, and closely related to those of '*Chlorobium ferrooxidans*' and *Chlorobium phaeobacteroides*. As already proposed (Imhoff, 1999), both species are transferred to the genus *Chlorobium*. Because *Pelodictyon clathratiforme* is the type species of the genus *Pelodictyon*, all members of the genus must be transferred to another genus. Because the different pigmentation, which was the main distinguishing feature between *Pelodictyon clathratiforme* and *Pelodictyon phaeoclathratiforme*, has been disregarded as a species-determinative property, and due to 16S rDNA sequence identity and lack of a type culture of *Pelodictyon clathratiforme*, it is proposed to combine both species and to consider strain DSM 5477^T as the type strain of *Chlorobium clathratiforme* comb. nov.

Emended description of *Chlorobium phaeovibrioides*

The species description is as given by Pfennig & Overmann (2001a), with the following modifications. Cells are curved rods or vibrioid, 0.3–0.7 µm wide, non-motile and occur as single cells. Colour of cell suspensions is green or brown, and photosynthetic pigments are BChl *d* (occasionally also BChl *c*) with chlorobactene as the major carotenoid in green strains, and BChl *e* with isorenieratene as the major carotenoid in brown strains. Photoautotrophic growth occurs with sulfide and sulfur as photosynthetic electron donors; thiosulfate may be used by some strains. In the presence of sulfide and bicarbonate, some simple organic compounds are photoassimilated. Vitamin B₁₂ is required for growth. Brackish water and marine bacteria that require at least 1 % NaCl. The DNA G+C content is 52–53.5 mol%.

The type strain is DSM 269^T. The GenBank/EMBL accession

number for the 16S rDNA sequence of the type strain is Y08105.

Comments. This species is a representative of group 2, according to Alexander *et al.* (2002). The type strain of *Chlorobium phaeovibrioides*, DSM 269^T, forms a distinct phylogenetic lineage which allows its recognition as a separate species. Almost-identical sequences of the 16S rRNA and *fmo* genes, salt requirement, similar fatty acid composition and DNA G+C contents of 52.0–53.5 mol% strongly support the inclusion of *Chlorobium phaeovibrioides* DSM 270, *Chlorobium vibrioforme* DSM 261 and *Chlorobium vibrioforme* subsp. *thiosulfatophilum* DSM 265 into this species. Strain DSM 265, which was previously considered as the type strain of *Chlorobium vibrioforme* subsp. *thiosulfatophilum*, loses type strain status and the name becomes a synonym of *Chlorobium phaeovibrioides*. Other strains of this subspecies must be assigned to different species (see below). It is particularly striking that the four strains now classified as *Chlorobium phaeovibrioides* were previously assigned to four different species and subspecies. Further studies are needed to prove the taxonomic status of *Chlorobium phaeovibrioides* UdG 6035, which is related to this species.

Description of *Chlorobium luteolum* comb. nov.

Basonym: *Pelodictyon luteolum* (Schmidle 1901) Pfennig and Trüper 1971 (Approved Lists 1980).

The species description is as given by Pfennig & Overmann (2001b) for *Pelodictyon luteolum*, with the following modifications. Cells are straight or curved rods or vibrioid, 0.6–0.9 µm wide, non-motile and may form gas vesicles. Colour of cell suspensions is green, and photosynthetic pigments are BChl *c* and *d* with chlorobactene as the major carotenoid. Photoautotrophic growth occurs with sulfide and sulfur as photosynthetic electron donors. In the presence of sulfide and bicarbonate, some simple organic compounds are photoassimilated. Vitamin B₁₂ is required for growth. Brackish water and marine bacteria that require at least 1 % NaCl. The DNA G+C content is 57.1–58.1 mol%.

The type strain is DSM 273^T. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is Y08107.

Comments. This species is a representative of group 2, according to Alexander *et al.* (2002). As outlined above, *Pelodictyon luteolum* must be assigned to another genus. As proposed by Imhoff (1999) and due to its phylogenetic relationship, the type strain of *Pelodictyon luteolum*, DSM 273^T, is transferred to the genus *Chlorobium* and becomes the type strain of the novel species *Chlorobium luteolum*. Almost-identical 16S rRNA and *fmo* gene sequences, together with a similar DNA G+C content (57.1 mol%, compared to 58.1 mol% of the type strain) strongly

support the inclusion of *Chlorobium vibrioforme* DSM 262 in this species.

Emended description of the genus *Prosthecochloris*

The genus *Prosthecochloris* comprises green sulfur bacteria with spherical to ovoid, vibrioid or rod-shaped cells that develop as single cells and may form non-branching prosthecae. Some species and strains may contain gas vesicles. The cell size ranges from 0.5 to 0.8 µm in width. As photosynthetic pigments, either BChl *c* and *d* or BChl *e* is present. The former are accompanied by carotenoids of the chlorobactene series, the latter by those of the isorenieratene series. Salt is required for growth. Vitamin B₁₂ is a growth factor. The genus comprises those strains and species that belong phylogenetically to group 1 of the green sulfur bacteria, according to Alexander *et al.* (2002). Photolithoautotrophic growth occurs under anoxic conditions in the light, with reduced sulfur compounds such as sulfide and elemental sulfur as electron donors. During oxidation of sulfide, elemental sulfur is transiently deposited outside cells. Final oxidation product is sulfate. In the presence of sulfide and bicarbonate, simple organic substrates are photoassimilated. Obligately phototrophic and strictly anaerobic. The DNA G+C content is 52.0–56.1 mol% (Bd).

The type species is *Prosthecochloris aestuarii* Gorlenko 1970, 148.

Emended description of *Prosthecochloris aestuarii*

The species description is as given by Gorlenko (2001). The type strain is DSM 271^T. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is Y07837.

Comments. The strain known as *Prosthecochloris aestuarii* 2K is genetically different from the type strain of *Prosthecochloris aestuarii*, to a degree that indicates species differentiation (97.3% 16S rDNA sequence similarity and significant differences in sequence signatures). It should be classified as a novel species of the genus *Prosthecochloris*. However, as no pure culture is presently available, this description is postponed.

Description of *Prosthecochloris vibrioformis* comb. nov.

Basonym: *Chlorobium vibrioforme* Pelsh 1936.

The species description is as given by Pfennig & Overmann (2001a) for *Chlorobium vibrioforme*, with the following modifications. Cells are curved rods or vibrioid, 0.5–0.7 µm wide, non-motile and occur as single cells. Gas vesicles may be formed. Colour of cell suspensions is green or brown, and photosynthetic pigments are BChl *d* (occasionally also BChl *c*) with chlorobactene as the major carotenoid in

green strains, and BChl *e* with isorenieratene as the major carotenoid in brown strains. Photoautotrophic growth occurs with sulfide and sulfur as photosynthetic electron donors; molecular hydrogen may be used by some strains. In the presence of sulfide and bicarbonate, some simple organic compounds are photoassimilated. Vitamin B₁₂ is required for growth by most strains. Brackish water and marine bacteria that require at least 1% NaCl. The DNA G+C content is 53.5 mol%.

The type strain is DSM 260^T. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is M62791.

Comments. The type strain of *Chlorobium vibrioforme*, DSM 260^T, does not belong to the genus *Chlorobium*, but is genetically closely related to *Prosthecochloris aestuarii*. The degree of relatedness justifies its recognition as a species of the genus *Prosthecochloris*. It is therefore proposed that strain DSM 260^T is recognized as the type strain of *Prosthecochloris vibrioformis* comb. nov. With this transfer, *Chlorobium vibrioforme* becomes a synonym of *Prosthecochloris vibrioformis*, and other strains assigned to this species, including subspecies, must be assigned to different species (see Table 1). Sequence similarities of more than 99% for both the 16S rRNA and the *fno* genes support the recognition of *Chlorobium phaeovibrioides* DSM 1678 and *Chlorobium vibrioforme* CHP 3402 as additional strains of *Prosthecochloris vibrioformis*.

Description of *Chlorobaculum* gen. nov.

Chlorobaculum (Chlo.ro.ba'cu.lum. Gr. adj. *chloros* green; L. n. *baculum* rod; N.L. neut. n. *Chlorobaculum* the green rod).

The genus *Chlorobaculum* comprises green sulfur bacteria with vibrioid or rod-shaped cells that develop singly. Some species and strains may contain gas vesicles. The cell size ranges from 0.3 to 1.1 µm in width. Photosynthetic pigments are BChl *c* and *d* or BChl *e*, with carotenoids of the chlorobactene series in green strains and of the isorenieratene series in brown strains. Sodium chloride may be required for growth. Vitamin B₁₂ is required as a growth factor by most species. The fatty acid composition is characterized by approximately 21–25% C_{14:0} and approximately 43% C_{16:1}. The genus comprises those strains and species that previously belonged to the genus *Chlorobium*, but form phylogenetic group 4 of the green sulfur bacteria, according to Alexander *et al.* (2002). Photolithoautotrophic growth occurs under anoxic conditions in the light, with reduced sulfur compounds such as sulfide, elemental sulfur and thiosulfate as electron donors. During oxidation of sulfide, elemental sulfur is transiently deposited outside cells. Final oxidation product is sulfate. In the presence of sulfide and bicarbonate, simple organic substrates are photoassimilated. Obligately phototrophic and strictly anaerobic. The DNA G+C content is 54.0–58.1 mol% (Bd).

The type species is *Chlorobaculum tepidum* comb. nov. (*Chlorobium tepidum* Wahlund *et al.* 1996, 1189).

Description of *Chlorobaculum tepidum* comb. nov.

Basonym: *Chlorobium tepidum* Wahlund *et al.* 1991.

The species description is basically that given by Wahlund *et al.* (1991) and Pfennig & Overmann (2001a) for *Chlorobium tepidum*. It is a moderately thermophilic freshwater species without a salt requirement and with a DNA G+C content of 56.5 mol%.

The type strain is ATCC 49652^T=DSM 12025^T. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is M58468.

Comments. The type strain of *Chlorobium tepidum*, ATCC 49652^T, is phylogenetically distant from all other strains that belong to this group. It is proposed as the type strain of *Chlorobaculum tepidum* sp. nov.

Description of *Chlorobaculum limnaeum* sp. nov.

Chlorobaculum limnaeum (lim.nae'um. Gr. adj. *limnaios* pertaining to or living in lakes; M.L. neut. adj. *limnaeum* living in lakes).

Cells are rod-shaped, 0.6–0.8 µm wide and non-motile. Colour of cell suspensions is brown or green, and photosynthetic pigments are BChl *e* with isorenieratene as the major carotenoid in brown strains and BChl *c* with chlorobactene as the major carotenoid in green strains. Photoautotrophic growth occurs with sulfide and sulfur as photosynthetic electron donors; molecular hydrogen may also be used. In the presence of sulfide and bicarbonate, some simple organic compounds are photoassimilated. Freshwater bacteria without a requirement for sodium chloride. Vitamin B₁₂ may be required for growth.

The type strain is DSM 1677^T. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is AJ290831.

Comments. Two strains that were known as *Chlorobium phaeobacteroides*, 1549 and DSM 1677, have almost-identical 16S rRNA and *fmo* gene sequences and are closely related to *Chlorobaculum tepidum*. They are phylogenetically distant from the type strain of *Chlorobium phaeobacteroides* and are assigned to *Chlorobaculum limnaeum* sp. nov. The strains known as *Chlorobium limicola* UdG 6038, UdG 6040, UdG 6042 and UdG 6045 have highly similar 16S rDNA sequences to the two strains of *Chlorobaculum limnaeum*, and may be considered as strains of this species. The assignment of strain UdG 6041 requires further study.

Description of *Chlorobaculum thiosulfatophilum* sp. nov.

Chlorobaculum thiosulfatophilum (thi.o.sul.fa.ti'phi.lum. N.L. n. *thiosulfas*, -atis thiosulfate; Gr. adj. *philos* loving; N.L. neut. n. *thiosulfatophilum* thiosulfate-loving).

Cells are rod-shaped, 0.7–1.1 µm wide, non-motile and occur as single cells. Colour of cell suspensions is green, and photosynthetic pigments are BChl *c* with chlorobactene as the major carotenoid. Photoautotrophic growth occurs with sulfide, sulfur and thiosulfate as photosynthetic electron donors; molecular hydrogen also may be used. In the presence of sulfide and bicarbonate, some simple organic compounds are photoassimilated. Freshwater bacteria without a requirement for sodium chloride. Vitamin B₁₂ may be required for growth. The DNA G+C content is 58.1 mol%.

The type strain is DSM 249^T. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is Y08102.

Comments. Two strains known as *Chlorobium limicola* subsp. *thiosulfatophilum*, DSM 249 and strain 1430, belong to group 4 of the green sulfur bacteria according to Alexander *et al.* (2002). They have a significantly higher DNA G+C content (58.1 mol%) than *Chlorobium limicola*, and cannot be considered to be subspecies of this species. They are considered to be strains of *Chlorobaculum thiosulfatophilum* sp. nov.

Description of *Chlorobaculum parvum* sp. nov.

Chlorobaculum parvum (par'vum. L. neut. adj. *parvum* small).

Cells are curved rods or vibrioid, 0.7–1.1 µm wide, non-motile and occur as single cells. Colour of cell suspensions is green, and photosynthetic pigments are BChl *d* with chlorobactene as the major carotenoid. Photoautotrophic growth occurs with sulfide, sulfur and thiosulfate as photosynthetic electron donors; molecular hydrogen may be used by some strains. In the presence of sulfide and bicarbonate, some simple organic compounds are photoassimilated. Vitamin B₁₂ may be required for growth. Brackish water and marine bacteria that require at least 1 % NaCl. The DNA G+C content is 56.1–56.6 mol%.

The type strain is DSM 263^T. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is Y10647.

Comments. Two strains, known as *Chlorobium vibrioforme* subsp. *thiosulfatophilum* DSM 263 (NCIB 8327, also referred to as *Chlorobium limicola* subsp. *thiosulfatophilum*) and NCIB 8346, are genetically closely related to each other but different from other known species. They have a characteristically high DNA G+C content (56.1 and 56.6 mol%, respectively) and require salt for optimum growth. They are phylogenetically distant from the type

strain of *Chlorobium vibrioforme* and belong to group 4 of the green sulfur bacteria, according to Alexander *et al.* (2002).

Description of '*Chlorobaculum chlorovibrioides*' comb. nov.

Basonym: *Chlorobacterium chlorovibrioides* Gorlenko *et al.* 1974.

The species description is as given by Pfennig & Overmann (2001a) for *Chlorobium chlorovibrioides*. Cells are curved rods or vibrioid, 0.3–0.4 µm wide, non-motile and occur as single cells. Colour of cell suspensions is green and photosynthetic pigments are BChl *d* with chlorobactene as the major carotenoid. Photoautotrophic growth occurs, with sulfide and sulfur as photosynthetic electron donors. In the presence of sulfide and bicarbonate, some simple organic compounds are photoassimilated. Vitamin B₁₂ is required for growth. Brackish water and marine bacteria that require at least 2–3% NaCl for optimal growth.

The type strain of *Chlorobium chlorovibrioides*, DSM 1377, is lost. A new type strain is currently not proposed. Strain UdG 6026 may qualify as such. The GenBank/EMBL accession number for the 16S rDNA sequence of this strain is Y10649.

Comments. The type strain of *Chlorobium chlorovibrioides*, DSM 1377 (DNA G+C content 54 mol%) has been lost, but a new isolate, UdG 6026, was assigned to this species (Figueras *et al.*, 1997). This strain belongs phylogenetically to the genus *Chlorobaculum* and is closely related to *Chlorobaculum parvum*. It is proposed as the type strain of '*Chlorobaculum chlorovibrioides*'. Based on 16S rDNA sequence data, strain *Chlorobium vibrioforme* UdG 6043 is closely related to UdG 6026 and is regarded as an additional strain of this species.

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