International Journal of Systematic Bacteriology (1998), **48**, 957–964 Printed in Great Britain

Taxonomic rearrangements of the genera *Thiocapsa* and *Amoebobacter* on the basis of 16S rDNA sequence analyses, and description of *Thiolamprovum* gen. nov.

Rémy Guyoneaud,[1] Jörg Süling,[2] Ralf Petri,[2] Robert Matheron,[3] Pierre Caumette,[1] Norbert Pfennig[4] and Johannes F. Imhoff[2]

Author for correspondence: Johannes F. Imhoff. Tel: +49 431 597 3850. Fax: +49 431 565876. e-mail: jimhoff@ifm.uni-kiel.de

[1] Laboratoire d'Océanographie Biologique, Université Bordeaux I, URA-CNRS 197, 2 rue du Professeur Jolyet, F-33120 Arcachon, France

[2] Institut für Meereskunde an der Universität Kiel, Abteilung Marine Mikrobiologie, Düsternbrooker Weg 20, D-24105 Kiel, Germany

[3] Laboratoire de Microbiologie, Faculté des Sciences et Techniques de Saint- Jérome, F-13397 Marseille Cedex 20, France

[4] Primelweg 12, D-88662, Überlingen, Germany

Complete nucleotide sequences of the 16S rDNAs were determined from *Thiocapsa* and *Amoebobacter* species, including all available type strains and some additional isolates. The distance-matrix analysis and the dendrogram for estimating the genetic relationships revealed that the investigated strains were found in two major clusters within the *Chromatiaceae*. One cluster comprises all *Amoebobacter* species, *Thiocapsa roseopersicina* and several isolates related to *Thiocapsa roseopersicina*. Representatives of the species *Amoebobacter roseus*, *Amoebobacter pendens* and *Thiocapsa roseopersicina*, the so called '*Thiocapsa roseopersicina* group', are very closely related, justifying their inclusion into one genus, *Thiocapsa*, for which an emended description is presented. *Amoebobacter purpureus* and *Amoebobacter pedioformis* formed two separate lines of descent with less than 93% (89·6–92·9%) similarity to strains of the '*Thiocapsa roseopersicina* group'. Therefore, they will be considered as two separate genera. As a consequence, an emended description is presented for the genus *Amoebobacter*, with *Amoebobacter purpureus* as the new type species and *A. pedioformis* is transferred to *Thiolamprovum pedioforme* gen. nov., comb. nov. Two species, *Thiocapsa pfennigii* and *Thiocapsa halophila*, which have been classified with the genus *Thiocapsa* because of their morphological properties, were found within another major cluster of the *Chromatiaceae* and are only distantly phylogenetically related to the first cluster with 88·4–90·6% and 90·4–92·2% sequence similarity, respectively.

Keywords: *Thiocapsa*, *Amoebobacter*, *Thiolamprovum*, *Chromatiaceae*, 16S rDNA sequences, genetic relationships, taxonomy

INTRODUCTION

Classification of *Chromatiaceae* (Bavendamm 1924, emend. Imhoff 1984) has been inherited from the morphological studies of Winogradsky (1888) and is still principally based on phenotypic characteristics. The first studies to establish genetic relationships of *Chromatiaceae* species were carried out on the basis of 16S rRNA oligonucleotide cataloguing (Fowler *et al*., 1984). They demonstrated that these bacteria are moderately related but form a coherent phylogenetic group for which the family name *Chromatiaceae* is justified. They also revealed strong discrepancies between phylogenetic relatedness and the taxonomic system of the family, based on phenotypic traits. Their study demanded more detailed investigations on the phylogenetic relationships within this family to establish a proper basis for a phylogenetically oriented taxonomy.

The first complete 16S rDNA sequences among the *Chromatiaceae* were obtained for *Chromatium vinosum* (De Weerd *et al*., 1990) and *Chromatium tepidum* (Wahlund *et al*., 1991). Recently, with the description

The EMBL accession numbers for the sequences reported in this paper are indicated in Table 1 and Fig. 1.

Table 1. Species and strains sequenced in this study

Previous name	New name	DSM no.	Original designation	EMBL accession no.	Reference
Thiocapsa roseopersicina	*Thiocapsa roseopersicina*	DSM 217^T	1711	Y12364	Pfennig (1989a, b), Pfennig & Trüper (1971a, b)
'*Thiocapsa roseopersicina*'	*Thiocapsa* sp.	–	9314	Y12303	Mandel *et al.* (1971)*
'*Thiocapsa roseopersicina*'	*Thiocapsa* sp.	–	10511	Y12300	Guyoneaud *et al.* (1997)†
'*Thiocapsa roseopersicina*'	*Thiocapsa* sp.	–	CE2209	Y12298	Guyoneaud *et al.* (1996)
'*Thiocapsa roseopersicina*'‡	*Thiocapsa* sp.	DSM 5653	5811	Y12301	Caumette *et al.* (1985)
'*Thiocapsa roseopersicina*'‡	*Thiocapsa* sp.	–	5812	Y12302	Caumette (1986)
Thiocapsa halophila	Uncertain affiliation	DSM 6210^T	SG3202	AJ002796	Caumette *et al.* (1991)
Thiocapsa pfennigii	Uncertain affiliation	DSM 226	8013	Y12373	Mandel *et al.* (1971)*
Amoebobacter pendens	*Thiocapsa* sp.	DSM 5652	5813	Y12396	Caumette *et al.* (1985)
Amoebobacter pendens	*Thiocapsa pendens*	DSM 236^T	1314	AJ002797	Pfennig (1989a, b), Pfennig & Trüper (1971a, b)
Amoebobacter roseus	*Thiocapsa rosea*	DSM 235^T	6611	AJ002798	Pfennig (1989a, b), Pfennig & Trüper (1971a, b)
Amoebobacter purpureus	*Amoebobacter purpureus*	DSM 4197^T	ThSchl2	Y12366	Eichler & Pfennig (1988)
Amoebobacter pedioformis	*Thiolamprovum pedioforme*	DSM 3802^T	CML2	Y12297	Eichler & Pfennig (1986)

* These strains have not been described phenotypically in the literature.

† Reference for 16S rDNA sequence.

‡ Tentatively designated as *Thiocapsa roseopersicina forma specialis* by Caumette *et al.* (1985).

of the new genera and species *Rhabdochromatium marinum* (Dilling *et al.*, 1995), *Chromatium glycolicum* (Caumette *et al.*, 1997) and *Thiorhodococcus minus* (Guyoneaud *et al.*, 1997), more 16S rDNA sequences became available, which confirmed the non-phylogenetic nature of the phenotypic classification of the *Chromatiaceae* (Guyoneaud *et al.*, 1997).

The genera *Thiocapsa* and *Amoebobacter* comprise the spherical and non-motile representatives of the *Chromatiaceae*. The differentiation between the two genera is based on the presence or absence of gas vesicles. In the case of *Ectothiorhodospiraceae* (Imhoff & Süling, 1996) and green sulfur bacteria (Overmann & Tuschack, 1997), the possession of gas vesicles is not considered to be of taxonomic relevance at the genus level. Moreover, the study based on 16S rRNA oligonucleotide cataloguing (Fowler *et al.*, 1984) had already revealed that some species of these two genera (*Thiocapsa roseopersicina*, *Amoebobacter pendens* and *Amoebobacter roseus*) are very closely related (S_{AB} values $\geqslant 0{\cdot}93$) and may actually comprise species of a single genus. Since this work, several new species have been described for both genera: *Amoebobacter pedioformis* (Eichler & Pfennig, 1986), *Amoebobacter purpureus* (Eichler & Pfennig, 1988) and *Thiocapsa halophila* (Caumette *et al.*, 1991). In addition, strains resembling *Thiocapsa roseopersicina* but containing okenone instead of spirilloxanthin as the major carotenoid were isolated (Caumette *et al.*, 1985). These new isolates were described and classified according to their phenotypic traits. Their genetic relationships have not been investigated so far. We have analysed the 16S rDNA sequences of the known species of the genera *Thiocapsa* and *Amoebobacter* (including all available type strains) and propose a taxonomic rearrangement at the genus level.

METHODS

Source and culture of bacterial strains. All *Thiocapsa* and *Amoebobacter* strains used for this study are listed in Table 1, which shows the previously used and the newly proposed names, the original strain designations, the DSM numbers (where available) and the EMBL accession numbers for their 16S rDNA sequences. Cultures of all strains are now maintained in our laboratories (see Table 1).

Strains were cultivated in a synthetic medium prepared anaerobically according to Pfennig & Trüper (1992). The medium contained: 0·03% KH_2PO_4; 0·05% NH_4Cl; 0·005% $CaCl_2.2H_2O$; 0·1% $MgCl_2.6H_2O$; 0·05% $MgSO_4.7H_2O$; 1 ml trace-element solution SL12 l^{-1} (Pfennig & Trüper, 1992); 0·02 mg vitamin B_{12} l^{-1}; 0·15% $NaHCO_3$; 0·05% $Na_2S.9H_2O$; final pH, 7·2. In addition, for some strains, 2% NaCl [strains 5811(=DSM 5653), 5812, 5813(=DSM 5652), CE2209] or 6% NaCl (strain DSM 6210) was added to the medium. Pure cultures were grown and maintained in 50 ml screw-capped bottles filled with synthetic medium.

PCR amplification and 16S rDNA sequencing and analysis. DNA for sequencing of 16S rRNA genes was obtained either from 1–2 ml well-grown liquid cultures or from freeze-dried material (*A. pedioformis* DSM 3802, *A. roseus* DSM 235 and *A. pendens* 5813). DNA was extracted and purified by using the QIAGEN genomic DNA buffer set. Recombinant *Taq* DNA polymerase was used for PCR (Mullis & Faloona, 1987) with the primers: 5′-GTTTGATCCTGGCTCAG-3′ and 5′-TACCTTGTTACGACTTCA-3′ (positions 11–27 and 1489–1506, respectively; according to the *Escherichia*

coli 16S rRNA numbering of the International Union of Biochemistry). The PCR products were purified by using the QIAquick PCR purification kit. Sequences were obtained by cycle sequencing with the SequiTherm sequencing kit (Biozym) and the chain termination reaction (Sanger *et al*., 1977) using an automated laser fluorescence sequencer (Pharmacia). Sequences were aligned using the CLUSTAL W program (Thompson *et al*., 1994). The alignment was from position 29–1381 according to the *Escherichia coli* numbering (including gaps, approx. 1400 positions). The distance matrix was calculated on the basis of the algorithm according to Jukes & Cantor (1969) with the DNADIST program within the PHYLIP package (Felsenstein, 1989). The FITCH program in the PHYLIP package fitted a tree to the evolutionary distances.

RESULTS AND DISCUSSION

Sequences of 16S rDNA from a number of strains of *Thiocapsa* and *Amoebobacter* species were determined, aligned and compared to those of other *Chromatiaceae*, *Ectothiorhodospira shaposhnikovii*, *Halorhodospira halophila* and *Escherichia coli*, which were available from the EMBL database. Sequence similarity and evolutionary distances (K_{nuc} values) are presented in Table 2; a dendrogram calculated on the basis of these values is shown in Fig. 1. The 16S rDNA gene sequence analysis confirmed that the representatives of the genera *Thiocapsa* and *Amoebobacter* are true members of the *Chromatiaceae*, placed within the gamma-*Proteobacteria*. Within the radiation of the family *Chromatiaceae*, the strains investigated were found in two clusters. Most of the strains formed one distinct cluster, which was not distinctly affiliated with one of the available reference organisms from this family (see Fig. 1). This cluster comprises all *Amoebobacter* species, *Thiocapsa roseopersicina* and strains related to this latter species. The analysis suggests a common ancestor of all of these strains and of *Chromatium vinosum* and related species. Two species were found within a second cluster, which includes '*Thiocapsa pfennigii*' and '*Thiocapsa halophila*' as well as *Rhabdochromatium marinum* and other marine *Chromatiaceae*.

Within the first cluster, *Thiocapsa roseopersicina*, *A. roseus* and *A. pendens* formed a closely related group (in the following referred to as the '*Thiocapsa roseopersicina* group') with a minimum of 93·9 % sequence similarity between the strains included in this study. These results are in agreement with the previous studies on 16S rRNA oligonucleotide cataloguing (Fowler *et al*., 1984), which already recognized the close relationship of these species (S_{AB} value of 0·93). *Amoebobacter purpureus* and *A. pedioformis*, however, formed two different lineages separated from the representatives of the '*Thiocapsa roseopersicina* group'. The sequence similarity between *A. purpureus*, *A. pedioformis* and all the other strains of this cluster was 89·6–91·9 and 88·9–92·9 %, respectively. Moreover, the sequence similarity between *A. purpureus* and *A. pedioformis* was 88·9 %, suggesting that these two species belong to two separate genera.

First of all, these results demonstrate a large phylogenetic distance between presently recognized species

Table 2. Levels of 16S rDNA sequence similarity and evolutionary distances of presently recognized *Thiocapsa* and *Amoebobacter* species with other phototrophic purple sulfur bacteria and *Escherichia coli* as reference species.

Alignment length was 1400 positions including gaps (bases 29–1381, according to *Escherichia coli* numbering). All strains were fitted to that size except for '*Thiocapsa roseopersicina*', DSM 217 (positions 69–1363 according to *Escherichia coli* numbering) and *A. roseus*, DSM 235^T (positions 71–1374 according to *Escherichia coli* numbering). The values on the upper right are the uncorrected percentages of sequence similarity; the values on the lower left are K_{nuc} values corrected for multiple base change by the method of Jukes & Cantor (1969).

Organism	Sequence similarity (%) and evolutionary distance (K_{nuc})																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 *Thiocapsa pfennigii* DSM 226^T		93·1	91·8	90·4	89·9	89·3	90·5	90·4	89·9	89·3	90·3	90·5	90.0	90·6	88·4	88·8	89·4	86·9	85·1	82·9
2 *Thiocapsa halophila* DSM 6210^T	0·073		94·5	92·4	92·2	90·8	91·7	91·1	91·1	90·4	92·2	92·0	92·1	92·2	91·1	91·5	91·5	89·9	87·2	84·2
3 *Rhabdochromatium marinum* DSM 5261^T	0·087	0·057		90·4	91·2	89·9	91·6	91·0	90·9	90·4	91·6	91·4	91·4	92·2	89·9	91·7	92·3	89·2	86·8	84·6
4 *Chromatium gracile* DSM 203^T	0·103	0·081	0·103		92·5	90·5	90·6	90·8	90·1	89·1	90·6	90·7	90·6	90·9	90·1	89·0	90·9	88·8	86·6	83·6
5 *Chromatium vinosum* DSM 180^T	0·108	0·083	0·094	0·079		91·8	92·2	91·0	91·1	91·0	92·5	92·4	92·4	92·8	89·9	90·7	94·3	89·4	86·6	84·3
6 *Thiocapsa roseopersicina* DSM 217^T	0·116	0·098	0·109	0·101	0·086		95·9	94·9	94·0	94·3	95·0	95·5	96·1	96·0	90·1	91·3	91·3	87·1	85·2	82·7
7 *Thiocapsa roseopersicina* 9314	0·101	0·088	0·089	0·100	0·082	0·042		95·8	94·8	95·1	96·3	96·3	95·7	96·4	90·9	92·4	92·4	88·7	86·4	83·4
8 *Amoebobacter pendens* DSM 236^T	0·103	0·095	0·095	0·098	0·096	0·053	0·043		94·9	94·5	95·7	95·9	95·5	95·8	91·9	91·1	91·8	88·2	85·0	82·7
9 *Amoebobacter pendens* DSM 5652	0·108	0·095	0·097	0·106	0·095	0·062	0·054	0·053		93·9	95·8	95·9	95·7	95·6	90·9	91·0	91·6	87·4	84·9	82·5
10 *Amoebobacter roseus* DSM 235^T	0·116	0·102	0·103	0·118	0·096	0·059	0·051	0·057	0·064		94·7	94·8	95·2	95·9	89·6	91·4	91·2	87·7	84·7	82·8
11 '*Thiocapsa roseopersicina*' DSM 5653	0·104	0·083	0·089	0·100	0·079	0·051	0·038	0·045	0·043	0·055		98·5	97·4	97·4	91·2	91·9	92·5	89·2	86·3	83·7
12 '*Thiocapsa roseopersicina*' 5812	0·102	0·084	0·091	0·100	0·080	0·047	0·038	0·042	0·042	0·054	0·015		97·3	97·6	91·5	92·0	92·1	89·0	85·8	83·1
13 '*Thiocapsa roseopersicina*' 10511	0·108	0·084	0·092	0·101	0·080	0·040	0·044	0·047	0·044	0·049	0·027	0·027		97·4	91·3	91·8	92·4	88·8	85·5	83·5
14 '*Thiocapsa roseopersicina*' CE2209	0·101	0·082	0·083	0·097	0·076	0·042	0·037	0·043	0·045	0·042	0·026	0·024	0·027		91·0	92·9	92·6	89·4	86·5	84·5
15 *Amoebobacter purpureus* DSM 4197^T	0·126	0·095	0·109	0·106	0·108	0·107	0·097	0·085	0·097	0·113	0·093	0·090	0·093	0·096		88·9	90·3	86·5	84·5	81·3
16 *Amoebobacter pedioformis* DSM 3802^T	0·121	0·091	0·088	0·119	0·099	0·093	0·081	0·095	0·096	0·091	0·085	0·085	0·086	0·075	0·120		91·6	87·7	84·1	83·0
17 *Thiocystis violacea* DSM 207^T	0·114	0·090	0·081	0·097	0·059	0·093	0·080	0·087	0·089	0·093	0·079	0.083	0·081	0·078	0·104	0·089		88·8	86·4	84·6
18 *Ectothiorhodospira shaposhnikovii* DSM 243^T	0·144	0·108	0·116	0·121	0·114	0·141	0·123	0·128	0·139	0·135	0·117	0·120	0·121	0·114	0·149	0·134	0·122		88·9	84·1
19 *Halorhodospira halophila* DSM 244^T	0·167	0·141	0·146	0·148	0·148	0·164	0·150	0·168	0·169	0·171	0·152	0·158	0·161	0·149	0·174	0·179	0·150	0·120		81·5
20 *Escherichia coli*	0·194	0·177	0·172	0·185	0·176	0·197	0·187	0·196	0·199	0·196	0·183	0·191	0·186	0·174	0·215	0·193	0·172	0·179	0·213	

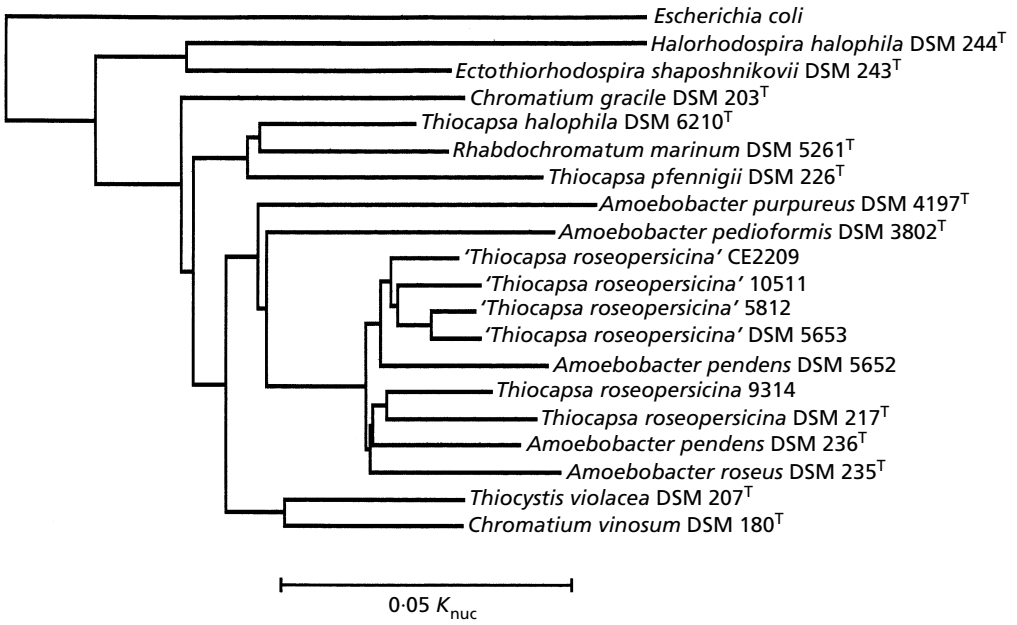

Fig. 1. Phylogenetic tree showing the relationships on the basis of 16S rDNA sequence similarity of strains belonging to the genera *Thiocapsa* and *Amoebobacter* together with other purple sulfur bacteria and *Escherichia coli* as reference organisms. Strain numbers and deposition numbers of the 16S rDNA sequences (in brackets) of reference strains not included in Table 1 are as follows: *Thiocystis violacea* DSM 207^T (Y11315), *Chromatium vinosum* DSM 180^T (M26629), *Chromatium gracile* DSM 203^T (X93473), *Rhabdochromatium marinum* DSM 5261^T (84316), *Ectothiorhodospira shaposhnikovii* DSM 243^T (M59151), *Halorhodospira halophila* DSM 244^T (M26630), *Escherichia coli* (K02555).

Table 3. Differential phenotypic traits of the genera *Thiocapsa*, *Amoebobacter* and *Thiolamprovum*

Data from references cited in Table 1. Bchl, bacteriochlorophyll; sp, spirilloxanthin; ok, okenone. Substrates used by all strains (+), some strains (+/−) or not used (−).

Characteristic	*Thiocapsa*	*Amoebobacter*	*Thiolamprovum*
Natural habitat	Water/mud from freshwater to marine environments	Stratified lakes	Wastewater ponds
Aggregate pattern	Tetrads, small irregular aggregates	Clumps of up to 40 cells	Platelets
Cell morphology	Spherical	Spherical/oval	Spherical/oval
Cell size (μm)	1·0–3·0	1·9–3·8 × 2·0–4·5	2·0 × 2·0–3·0
Gas vesicles	+/−	+	+
Bchl/carotenoid	Bchl *a*/sp, ok	Bchl *a*/ok	Bchl *a*/sp
Internal membranes	Vesicular	Vesicular	Vesicular
G+C content (mol%)	63·3–66·3	63·4–64·1	64·5–66·5
Substrates used:			
Sulfide	+	+	+
Thiosulfate	+	+	+
Propionate	+	+	−
Pyruvate	+	+	+
Malate	+	−	−

Table 4. Differential phenotypic traits of the type strains of the recognized species of the genus *Thiocapsa*

Data from references given in Table 1 and this study. See Table 3 legend for symbols and abbreviations

Characteristic	*Thiocapsa roseopersicina* (DSM 217^T)	*Thiocapsa pendens* (DSM 236^T)	*Thiocapsa rosea* (DSM 235^T)
Cell morphology	Spherical	Spherical	Spherical
Cell size (μm)	1·0–3·0	1·5–2·0	2·0–3·0
Gas vesicles	No	Yes	Yes
Bchl/carotenoid	Bchl *a*/sp	Bchl *a*/sp	Bchl *a*/sp
G+C content (mol%)	65·3	65·3	64·3
SO_4^{2-}-assimilation	+	−	−
Chemotrophic growth	+	−	+
Substrates used:			
Hydrogen	+	−	−
Sulfide	+	+	+
Thiosulfate	+	+	+
Formate	−	−	−
Acetate	+	+	+
Propionate	+	+	+
Pyruvate	+	+	+
Malate	+	+	−
Succinate	+	−	−
Fumarate	+	−	−
Glucose	−	+	−
Fructose	+	−	+
Glycerol	+	−	−

of the genus *Thiocapsa*; *Thiocapsa roseopersicina* (the type species), *Thiocapsa pfennigii* and *Thiocapsa halophila*, which have been classified into the genus *Thiocapsa* on the basis of morphological properties (non-motile cocci with internal sulfur globules). The sequence differences, however, do not merit the grouping of these species within one genus. Therefore, *Thiocapsa pfennigii* and *Thiocapsa halophila* have to be removed from the genus *Thiocapsa*, the name of which will stay with the type species, *Thiocapsa roseopersicina*. A formal taxonomic transfer will not be proposed at this stage, because the exact relationship of these two bacteria with other members of the *Chromatiaceae* is presently not established.

The second major consequence of our results is the close phylogenetic relationships between *Thiocapsa roseopersicina* and *A. roseus* as well as other *Amoebobacter* species. *Thiocapsa roseopersicina* may have evolved from an ancestor containing gas vesicles by loss of this property; some strains may still contain genes for the production of gas vesicles and may even be able to form such vesicles under certain, so far unrecognized, conditions. Nonetheless, it is obvious that the formation of gas vesicles is not of taxonomic relevance at the genus level. Other phenotypic features have to be considered to separate *A. purpureus*, *A. pedioformis* and representatives of the '*Thiocapsa roseopersicina* group'. Morphological traits such as cell morphology, aggregate patterns and the presence or absence of a strong slime capsule (Table 3) may be considered for characterizing the species. Therefore, on the basis of genetic and phenotypic properties, we propose to maintain the genus *Amoebobacter*, with *A. purpureus* as the new type species and to transfer *A. pedioformis* to *Thiolamprovum pedioforme* gen. nov., comb. nov.

The '*Thiocapsa roseopersicina* group' is represented by three species: *Thiocapsa roseopersicina*, *A. pendens* and *A. roseus*. The strains studied do form two sub-groups, corresponding to the three type strains of the species and a second group of isolates tentatively assigned to *Thiocapsa roseopersicina* and *A. pendens*, respectively (Table 1, Fig. 1).

Apart from the formation of gas vesicles, which could be of taxonomic importance at the species level, some physiological features such as substrate utilization and chemolithoautotrophic growth capacities separate the three existing species (Table 4). All strains of *Thiocapsa roseopersicina* characteristically use hydrogen, glycerol, fructose, succinate, fumarate and malate as substrates. *A. pendens* and *A. roseus* do not use hydrogen, glycerol, succinate or fumarate (Table 4). In addition, *A. roseus* does not use malate, whereas *A.*

pendens is the only species able to use glucose but not fructose. *Thiocapsa roseopersicina* and *A. roseus* can grow chemolithoautotrophically with oxygen in the dark (De Wit & van Gemerden, 1987; Kämpf & Pfennig, 1980; Overmann & Pfennig, 1992). The two strains classified as *A. pendens* exibited differences with regard to chemotrophic growth. *A. pendens* DSM 236^T is unable to grow chemotrophically (Kämpf & Pfennig, 1980) whereas '*Amoebobacter pendens*' DSM 5652 grows chemolithoautotrophically (Overmann & Pfennig, 1992). This strain had been originally and tentatively identified as a *Thiocapsa roseopersicina* (Caumette *et al*., 1985), but was later reclassified as *A. pendens* (Eichler & Pfennig, 1986), although it was different from the type strain of this species with regard to chemotrophic growth and gas vesicles were not always present.

Because 'it is the presence or absence of phenotypic coherency among strains that should be the deciding factor' (Stackebrandt & Goebel, 1994), we propose to maintain these three existing species and to consider them as members of the genus *Thiocapsa*, with *Thiocapsa roseopersicina* as the type species. Therefore, we propose to transfer *A. pendens* to *Thiocapsa pendens* comb. nov. and *A. roseus* to *Thiocapsa rosea* comb. nov.

Within this genus, strains 5811 (DSM 5653) and 5812 were mentioned as *Thiocapsa roseopersicina forma specialis* (Caumette *et al*., 1985), because they contain okenone as the major carotenoid, while the other phenotypic traits were identical to those of *Thiocapsa roseopersicina* (Table 4). They are closely related genetically (Table 2), and could probably be described as a new species within the genus *Thiocapsa* on the basis of genetic relationship and pigment composition. Nevertheless, they both are closely related to the *Thiocapsa* sp. strain 10511 (97·4 and 97·3% sequence similarity, respectively), which contains spirilloxanthin as the main carotenoid. A decision at the species level would require DNA–DNA reassociation studies and is therefore not proposed at the present level of our knowledge. A similar uncertainty that could possibly be resolved by hybridization studies is the exact species assignment of other strains, which have been tentatively identified as belonging to *Thiocapsa roseopersicina* (CE2209, 9314, 10511) and *A. pendens* (DSM 5652). However, knowledge of genetic relationships and phenotypic features undoubtedly permit an assignment of these strains to the genus *Thiocapsa*.

Emended descriptions of the genera *Thiocapsa* and *Amoebobacter* are given, and the following taxonomic changes are proposed: transfer of *Amoebobacter roseus* (the former type species of the genus *Amoebobacter*) to the genus *Thiocapsa* and description as a new combination, *Thiocapsa rosea* comb. nov.; transfer of *Amoebobacter pendens* to the genus *Thiocapsa* and description as a new combination, *Thiocapsa pendens* comb. nov.; definition of *Amoebobacter purpureus* as the new type species of the genus *Amoebobacter*; removal of *Thiocapsa pfennigii* and *Thiocapsa halophila* from the genus *Thiocapsa*; and transfer of *Amoebobacter pedioformis* to the new genus *Thiolamprovum* gen. nov. as *Thiolamprovum pedioforme* comb. nov.

Emended description of the genus *Thiocapsa* Winogradsky 1888, 84[AL]

Thiocapsa (Thi.o.cap′sa. Gr. n. *thios* sulfur; L. n. *capsa* box; M.L. fem. n. *Thiocapsa* sulfur box).

Cells are spherical, 1·0–3·0 μm in diameter, diplococci before multiplication by binary fission and are non-motile. Tetrads may be formed after consecutive division in two perpendicular planes. Individual cells are surrounded by a strong slime capsule. May contain gas vesicles. Gram-negative. Internal photosynthetic membrane system of vesicular type containing the photosynthetic pigments bacteriochlorophyll *a* and carotenoids. Phototrophic under anoxic conditions in the light, may be chemoautotrophic or mixotrophic under micro-oxic to oxic conditions in the dark. Capable of photolithoautotrophic growth with sulfide, thiosulfate and sulfur as electron donor. Elemental sulfur globules are transiently stored inside the cells, final oxidation product is sulfate. May require vitamin B_{12}. The G + C content of the DNA is 63·3–66·3 mol% (Bd). Type species is *Thiocapsa roseopersicina*.

Description of *Thiocapsa rosea* comb. nov. (*Amoebobacter roseus* Winogradsky 1888, 77[AL])

Thiocapsa rosea (ro′se.a. L. adj. *rosea* rosy, rose-coloured, pink).

The description is the same as that given by Winogradsky (1888) and Pfennig (1989b). Neotype strain is DSM 235 (= strain 6611, Davis).

Description of *Thiocapsa pendens* comb. nov. (*Amoebobacter pendens* Pfennig and Trüper 1971, 13[AL]; *Rhodothece pendens* Molisch 1906, 230)

Thiocapsa pendens (pen′dens. L. part. adj. *pendens* hanging).

The description is the same as that given by Pfennig, (1989b) and Pfennig & Trüper (1971a). Neotype strain is DSM 236 (= strain 1314, Klein-Kalden).

Emended description of the genus *Amoebobacter* Winogradsky 1888, 71[AL]

Amoebobacter (A.moe.bo.bac′ter. Gr. n. *amoebe* change, transformation; M.L. n. *bacter* a rod; M.L. masc. n. *Amoebobacter* changeable rod).

Cells are nearly spherical to oval, 1·9–3·8 × 2·0–4·5 μm in size, may occur in irregular aggregates of up to 40 cells, multiplication by binary fission, non-motile, Gram-negative. Internal photosynthetic membrane system of vesicular type containing the photosynthetic

pigments bacteriochlorophyll *a* and carotenoids. Phototrophic under anoxic conditions in the light, may be chemoautotrophic or mixotrophic under micro-oxic conditions in the dark. Capable of photolithoautotrophic growth with sulfide, thiosulfate and elemental sulfur as electron donor, elemental sulfur globules are transiently stored inside the cells, final oxidation product is sulfate. Assimilatory sulfate reduction lacking. The G+C content of the DNA is 63·4–64·1 mol% (Bd). Type species is *Amoebobacter purpureus*.

Description of *Amoebobacter purpureus* Eichler and Pfennig 1988

Amoebobacter purpureus (pur.pur′e.us. L. masc. adj. *purpureus* purple or purple-red).

The description is the same as that given by Eichler & Pfennig (1988). Type strain is DSM 4197^T (=strain ThSchl2^T, =Schleinsee^T).

Description of *Thiolamprovum* gen. nov.

Thiolamprovum (Thi.o.lam.pro′vum. Gr. n. *thios* sulfur; Gr. n. *lampros* bright, brilliant; L. n. *ovum* egg; M.L. masc. n. *Thiolamprovum* bright egg with sulfur).

Cells nearly spherical to oval, 2 × 2–3 μm in size, may occur in regular platelets of 4–16 cells, multiplication by binary fission, non-motile, Gram-negative. Internal photosynthetic membrane system of vesicular type containing the photosynthetic pigments bacteriochlorophyll *a* and carotenoids. Phototrophic under anoxic conditions, may be chemoautotrophic or mixotrophic under micro-oxic conditions in the dark. Capable of photolithoautotrophic growth with sulfide, thiosulfate and elemental sulfur as electron donor, elemental sulfur globules are transiently stored inside the cells, final oxidation product is sulfate. Assimilatory sulfate reduction lacking. The G+C content of the DNA is 64·5–66·5 mol% (Bd). Type species is *Thiolamprovum pedioforme*.

Description of *Thiolamprovum pedioforme* comb. nov. (*Amoebobacter pedioformis* Eichler and Pfennig 1986)

Thiolamprovum pedioforme (pe.di.o.for′me. Gr. n. *pedion* a plain, a flat area; L. n. *forma* shape; M.L. neut. adj. *pedioforme* flat-shaped).

The description is the same as that given by Eichler & Pfennig (1986). Type strain is DSM 3802^T (=strain CML2^T, =Taichung^T).

REFERENCES

Bavendamm, W. (1924). Die farblosen und roten Schwefelbakterien. Fischer, Jena.

Caumette P. (1986). Phototrophic sulfur bacteria and sulfate-reducing bacteria causing red waters in a shallow brackish coastal lagoon (Prévost Lagoon, France). *FEMS Microbiol Ecol* **38**, 113–124.

Caumette, P., Schmidt, K., Biebl, H. & Pfennig, N. (1985). Characterization of a *Thiocapsa* strain containing okenone as major carotenoid. *Syst Appl Microbiol* **6**, 132–136.

Caumette, P., Baulaigue, R. & Matheron, R (1991). *Thiocapsa halophila* sp. nov., a new halophilic phototrophic purple sulfur bacterium. *Arch Microbiol* **155**, 170–176.

Caumette, P., Imhoff, J. F., Süling, J. & Matheron, R. (1997). *Chromatium glycolicum* sp. nov., a moderately halophilic purple sulfur bacterium that uses glycolate as substrate. *Arch Microbiol* **167**, 11–18.

De Weerd, K. A., Mandelco, L., Tanner, R. S., Woese, C. R. & Suflita, J. M. (1990). *Desulfomonile tiedjei* gen. nov. and sp. nov., a novel anaerobic, dehalogenating, sulfate-reducing bacterium. *Arch Microbiol* **154**, 23–30.

De Wit, R. & van Gemerden, H. (1987). Chemolithotrophic growth of the phototrophic purple sulfur bacterium *Thiocapsa roseopersicina*. *FEMS Microbiol Ecol* **45**, 117–126.

Dilling, W., Liesack, W. & Pfennig, N. (1995). *Rhabdochromatium marinum* gen. nom. rev., sp. nov., a purple sulfur bacterium from salt marsh microbial mat. *Arch Microbiol* **164**, 125–131.

Eichler, B. & Pfennig, N. (1986). Characterization of a new platelet-forming purple sulfur bacterium, *Amoebobacter pedioformis* sp. nov. *Arch Microbiol* **146**, 295–300.

Eichler, B. & Pfennig, N. (1988). A new purple sulfur bacterium from stratified freshwater lakes, *Amoebobacter purpureus* sp. nov. *Arch Microbiol* **149**, 395–400.

Felsenstein, J. (1989). PHYLIP – phylogenetic inference package (version 3.2). *Cladistics* **5**, 164–166.

Fowler, V. J., Pfennig, N., Schubert, W. & Stackebrandt, E. (1984). Towards a phylogeny of phototrophic bacteria – 16S rRNA oligonucleotide cataloguing of 11 species of *Chromatiaceae*. *Arch Microbiol* **139**, 382–387.

Guyoneaud, R., Matheron, R., Baulaigue, R., Podeur, K., Hirschler, A. & Caumette, P. (1996). Anoxygenic phototrophic bacteria in eutrophic coastal lagoons of the French Mediterranean and Atlantic coasts (Prévost Lagoon, Arcachon Bay, Certes Fishponds). *Hydrobiologia* **329**, 33–43.

Guyoneaud, R., Matheron, R., Liesack, W., Imhoff, J. F. & Caumette, P. (1997). *Thiorhodococcus minus* gen. nov., sp. nov., a new purple sulfur bacterium isolated from coastal lagoon sediments. *Arch Microbiol* **168**, 16–23.

Imhoff, J. F. (1984). Reassignment of the genus *Ectothiorhodospira* Pelsh 1936 to a new family *Ectothiorhodospiraceae* fam. nov., and emended description of the *Chromatiaceae* Bavendamm 1924. *Int J Syst Bacteriol* **34**, 338–339.

Imhoff, J. F. & Süling, J. (1996). The phylogenetic relationship among *Ectothiorhodospiraceae*. A re-evaluation of their taxonomy on the basis of rDNA analyses. *Arch Microbiol* **165**, 106–113.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. M. Munro. New York: Academic Press.

Kämpf, C. & Pfennig, N. (1980). Capacity of *Chromatiaceae* for chemotrophic growth. Specific respiration rates of *Thiocystis violacea* and *Chromatium vinosum*. *Arch Microbiol* **127**, 125–135.

Mandel, M., Leadbetter, E. R., Pfennig, N. & Trüper, H. G. (1971). Deoxyribonucleic acid base compositions of phototrophic bacteria. *Int J Syst Bacteriol* **21**, 222–230.

Mullis, K. B. & Faloona, F. (1987). Specific synthesis of DNA in vitro via a polymerase-catalysed chain reaction. *Methods Enzymol* **155**, 335–350.

Overmann, J. & Pfennig, N. (1992). Continuous chemotrophic growth and respiration of *Chromatiaceae* species at low oxygen concentrations. *Arch Microbiol* **158**, 59–67.

Overmann, J. & Tuschak, C. (1997). Phylogeny and molecular fingerprinting of green sulfur bacteria. *Arch Microbiol* **167**, 302–309.

Pfennig, N. (1989a). Genus IV. *Thiocapsa* Winogradsky 1888, 84^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 1645–1647. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Willkins.

Pfennig, N. (1989b). Genus VIII. *Amoebobacter* Winogradsky 1888, 71^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 1651–1652. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Willkins.

Pfennig, N. & Trüper, H. G. (1971a). New nomenclatural combinations in the phototrophic sulfur bacteria. *Int J Syst Bacteriol* **21**, 11–14.

Pfennig, N. & Trüper, H. G. (1971b). Type and neotype strains of the phototrophic bacteria maintained in pure culture. *Int J Syst Bacteriol* **21**, 19–24.

Pfennig, N. & Trüper H. G. (1992). The family *Chromatiaceae*. In *The Prokaryotes* pp. 3200–3221. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

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

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Wahlund, T. M., Woese, C. R., Castenholz, R. W. & Madigan, M. T. (1991). A thermophilic green sulfur bacterium from New Zealand hot springs, *Chlorobium tepidum* sp. nov. *Arch Microbiol* **156**, 81–90.

Winogradsky, S. (1888). Beiträge zur Morphologie und Physiologie der Bakterien, Heft 1. Zur Morphologie und Physiologie der Schwefelbakterien. Leipzig: Felix.