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Thiocapsa marina sp. nov., a novel, okenone-containing, purple sulfur bacterium isolated from brackish coastal and marine environments

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Four marine, phototrophic, purple sulfur bacteria (strains 5811^T, 5812, BM-3 and BS-1) were isolated in pure culture from different brackish to marine sediments in the Mediterranean Sea, the White Sea and the Black Sea. Single cells of these strains were coccus-shaped, non-motile and did not contain gas vesicles. The colour of cell suspensions that were grown in the light was purple–red. Bacteriochlorophyll *a* and carotenoids of the okenone series were present as photosynthetic pigments. Photosynthetic membrane systems were of the vesicular type. Hydrogen sulfide, thiosulfate, elemental sulfur and molecular hydrogen were used as electron donors during photolithotrophic growth under anoxic conditions; carbon dioxide was utilized as the carbon source. During growth on sulfide, elemental sulfur globules were stored inside the cells. In the presence of hydrogen sulfide, several organic substances could be photoassimilated. Comparative 16S rDNA sequence analysis revealed an affiliation of these four strains to the genus *Thiocapsa*. Both phylogenetic analysis and the results of DNA–DNA hybridization studies revealed that these strains formed a separate cluster within the genus *Thiocapsa*. Thus, according to phenotypic characteristics and mainly the carotenoid composition, 16S rDNA sequence analysis and DNA–DNA hybridization data, it is proposed that these strains should be classified as a novel species, *Thiocapsa marina* sp. nov., with strain 5811^T (=DSM 5653^T=ATCC 43172^T) as the type strain.

The genus *Thiocapsa* currently comprises four species, including the type species *Thiocapsa roseopersicina*, two species that were originally described as members of the genus *Amoebobacter*, i.e. *Thiocapsa rosea* and *Thiocapsa pendens* (Guyoneaud *et al.*, 1998), and the more recently described *Thiocapsa litoralis* (Puchkova *et al.*, 2000). In contrast, two species that were previously described as members of the genus *Thiocapsa* are now recognized as members of separate genera, i.e. *Thiococcus pfennigii* and *Thiohalocapsa halophila* (Imhoff *et al.*, 1998).

During ecological investigations in the brackish coastal lagoon 'Etang du Prevost' (French Mediterranean coast), a particular purple–red, okenone-containing strain of *Thiocapsa* (strain 5811^T) was isolated (Caumette, 1986) and characterized according to its phenotypic characteristics (Caumette *et al.*, 1985). At that time, it was concluded that it would be premature to consider strain 5811^T as a novel species solely on the basis of differences in pigment composition. Subsequently, other okenone-containing *Thiocapsa* strains (strains 5812, BS-1 and BM-3) were isolated from different environments (Prevost Lagoon, Romanian coast of the Black Sea and Russian coast of the White Sea, respectively). In this paper, on the basis of cytological, biochemical, physiological and genetic properties, it is proposed that these four strains should be classified as members of a novel species, *Thiocapsa marina* sp. nov., with strain 5811^T (=DSM 5653^T=ATCC 43172^T) as the type strain.

Strains 5811^T and 5812 were isolated from Prevost Lagoon (French Mediterranean coast) during red-water periods in July 1977 and 1979 (Caumette, 1986). During the time of sampling, the water was anoxic, contained high sulfide concentrations (up to 3–4 mM) and had a salinity of 2·0–3·5 %. Strain BS-1 was isolated from hydrogen

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sulfide-containing sediment in the Black Sea near the coast of Romania, at a depth of 30 m. Strain BM-3 was isolated from a microbial mat located in a natural, shallow, supralittoral beach, which was only periodically covered by sea water, on the Russian coast of the White Sea. For isolation of strains, the culture medium, prepared according to Pfennig & Trüper (1992), contained [(l distilled water)$^{-1}$]: KH_2PO_4, 0·3 g; NH_4Cl, 0·5 g; $CaCl_2.2H_2O$, 0·05 g; $MgCl_2.6H_2O$, 1 g; $MgSO_4.7H_2O$, 0·5 g; NaCl, 20 g; trace element solution SL12 (Overmann *et al.*, 1992), 1 ml; vitamin B_{12}, 0·02 mg; $NaHCO_3$, 1·5 g; and $Na_2S.9H_2O$, 0·5 g. The final pH was adjusted to 7·2–7·4. Pure cultures were obtained as described by Guyoneaud *et al.* (1997). Microscopic observations, absorption spectra of living cells and acetone extracts were realized according to Caumette *et al.* (1985) and Caumette (1986). Growth tests for substrate utilization, optimum NaCl concentration, pH and temperature and light intensity requirements were performed according to Guyoneaud *et al.* (1997). Semi-aerobic growth, vitamin requirements, capacity for assimilatory sulfate reduction and capacity for dinitrogen fixation were tested according to Guyoneaud *et al.* (1997).

Individual cells of the four purple–red strains were non-motile cocci, 1·5–3·0 μm in diameter. They were similar morphologically to *Thiocapsa roseopersicina* and *Thiocapsa litoralis*, but differed from *Thiocapsa pendens* and *Thiocapsa rosea* by their lack of gas vesicles. The four strains contained bacteriochlorophyll *a* and okenone as main pigments. In the near-infrared region of the spectra, the major peak of bacteriochlorophyll *a* was 825–830 nm, whereas the usual 800 nm peak was very low, appearing as a shoulder on the major peak (Fig. 1b). The broad peak at 520–525 nm is characteristic of the presence of okenone as the main carotenoid. In comparison, spirilloxanthin-containing *Thiocapsa roseopersicina* has two major peaks in the near-infrared region around 800 and 860 nm (Fig. 1a). Chemical carotenoid analysis of strains 5811^T, 5812 and BM-3 confirmed okenone as the main carotenoid (Caumette *et al.*, 1985; Sidorova *et al.*, 1998). However, results revealed the presence of three carotenoid series in these strains. In addition to okenone, which is also the major carotenoid, spirilloxanthin and the ketocarotenoids found in *Rhodopila globiformis* were also present. No modification in carotenoid composition was detected when growth occurred under different culture conditions. This peculiar pigment composition is the most obvious phenotypic characteristic that distinguishes these four purple–red strains from other *Thiocapsa* species.

The four okenone-containing strains are rather similar to *Thiopcapsa roseopersicina*, according to substrate utilization and other major physiological properties. Electron donors and/or carbon sources used by the novel strains during photolithotrophic and photo-organotrophic growth were as follows: sulfide, sulfite, sulfur, thiosulfate, molecular hydrogen, acetate, formate, propionate, pyruvate, malate, fumarate, succinate, lactate, fructose, glycerol, peptone,

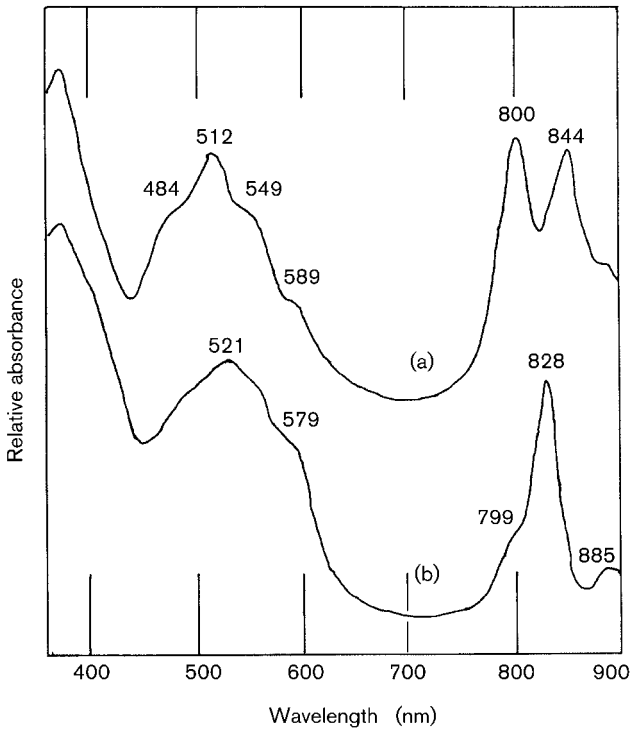

Fig. 1. Absorption spectra of whole cells of spirilloxanthin-containing *Thiocapsa roseopersicina* (a) and okenone-containing *Thiocapsa marina* strain 5811^T (b).

Casamino acids and yeast extract. The following substrates (concentration in mM) were tested, but not utilized: dimethylsulfide (2), thioglycolate (2), thioacetamide (2), cysteine (2), methionine (2), aspartate (5), glutamate (5), glycine betaine (2), 2-oxoglutarate (5), crotonate (5), caprylate (2), pelargonate (2), palmitate (2), cyclohexane carboxylate (2), benzoate (2), tartrate (2), nicotinate (2), catechol (2), ascorbate (2), gallate (2), gluconate (5), trehalose (2) and *N*-acetylglucosamine (2). All four strains could grow chemolithotrophically and chemoorganotrophically under micro-oxic conditions in the dark. No anaerobic growth was observed in the dark. The four strains were isolated from marine environments and showed NaCl optima of 1–2 % and considerable tolerance up to 8 % NaCl.

DNA was isolated by the method of Marmur (1961). The DNA base composition was determined according to Owen *et al.* (1969). DNA–DNA hybridizations were performed according to standard procedures (De Ley *et al.*, 1970). Cell material for 16S rDNA sequencing was taken from 1–2 ml well-grown liquid cultures. DNA was extracted and purified by using a QIAamp DNA extraction kit (Qiagen). PCR amplification and 16S rDNA sequencing were done as described previously (Imhoff *et al.*, 1998; Imhoff & Pfennig, 2001). Sequences were aligned by using the program CLUSTAL_W (Thompson *et al.*, 1994) and corrected

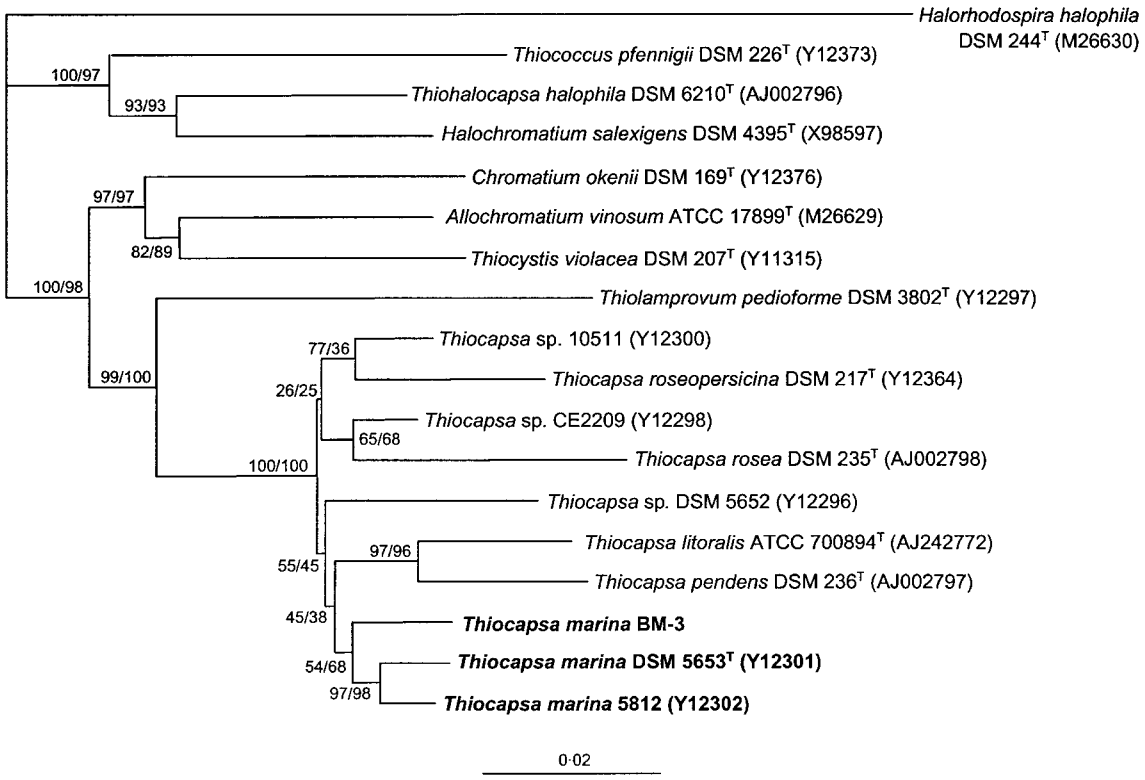

Fig. 2. Phylogenetic tree using 16S rDNA sequences of representatives of the *Chromatiaceae* with special emphasis on *Thiocapsa* species and related genera. The tree demonstrates the formation of a distinct cluster by the purple–red strains described in this communication, which is located within the *Thiocapsa* branch. Calculations were made based on alignments using CLUSTAL_W. Distance methods using DNADIST, FITCH and DNAML were used for maximum-likelihood calculations (PHYLIP package). Bootstrap analyses with 100 resamplings were performed to obtain estimates for phylogenetic tree topologies; the two numbers given at nodes indicate analyses based on distance matrices and maximum-likelihood calculations, respectively. Strain designations and 16S rDNA GenBank accession numbers are indicated. Bar, 2 % difference in nucleotide sequence.

manually. A distance matrix was calculated on the basis of the F84-algorithm according to Kishino & Hasegawa (1989), with the program DNADIST within the PHYLIP package (Felsenstein, 1989). The program FITCH (Fitch & Margoliash, 1967) in the PHYLIP package fitted a tree to the evolutionary distances. Phylogenetic trees calculated by maximum-likelihood were constructed by the program DNAML (Felsenstein & Churchill, 1996) of the PHYLIP package. Parameter G (global rearrangement) was invoked and J (jumble) was set to 10 in all calculations (see PHYLIP documentation). Phylogenetic trees based on distance matrices and maximum-likelihood calculations were bootstrapped with 100 resamplings.

The DNA G + C contents of the four isolates were 62·7, 62·9, 62·9 and 63·2 mol% for strains 5811^T, 5812, BS-1 and BM-3, respectively; these values are lower than those of other *Thiocapsa* species. Caumette *et al.* (1985) already suggested that the purple–red strains 5811^T and 5812 may be considered as subspecies or *forma specialis* of *Thiocapsa roseopersicina*. These authors considered analysis of the genetic relatedness between purple–red and rose-red strains of *Thiocapsa roseopersicina* to be crucial for a definite assignment of the purple–red strains. More recently, taxonomic rearrangement of the genera *Thiocapsa* and *Amoebobacter* on the basis of 16S rDNA sequence analysis (Guyoneaud *et al.*, 1998) revealed that both strains 5811^T and 5812 were not related closely to the type strains of *Thiocapsa roseopersicina*, *Thiocapsa rosea* or *Thiocapsa pendens*. Comparative 16S rDNA sequence analysis revealed a common ancestor between the four novel strains and the type strains of recognized *Thiocapsa* species (Fig. 2). No close relationship was found between the novel isolates and *Thiohalocapsa halophila*, which also represents a coccoid, purple sulfur bacterium from marine and saline environments and contains okenone as the main carotenoid (Caumette *et al.*, 1991). Within the genus *Thiocapsa*, all four strains formed a tight cluster, with 16S rDNA sequence similarities of >99 %. This suggests that these strains could be recognized as representatives of a novel species of the genus *Thiocapsa*.

Results of DNA–DNA reassociation studies confirmed this result and revealed high DNA relatedness between the four

Table 1. DNA G+C contents and DNA–DNA reassociation between *Thiocapsa roseopersicina* DSM 217^T, *Thiohalocapsa halophila* DSM 6210^T and the four purple–red strains of *Thiocapsa marina* described in this study

Strain	G+C content (mol%)	Degree of DNA–DNA reassociation (%) with DNA from strain:				
		1	2	3	4	5
1. *Thiocapsa roseopersicina* DSM 217^T	63·3	100				
2. *Thiocapsa marina* BS-1	62·9	45	100			
3. *Thiocapsa marina* BM-3	63·2	45	70	100		
4. *Thiocapsa marina* 5811^T	62·7	47	87	75	100	
5. *Thiocapsa marina* 5812	62·9	41	81	ND	86	100
6. *Thiohalocapsa halophila* DSM 6210^T	66·3	10	ND	ND	14	ND

ND, Not determined.

strains (70–87 %). In contrast, all four strains had low DNA relatedness with the type strains of *Thiocapsa roseopersicina* and *Thiohalocapsa halophila* (Table 1). *Thiohalocapsa halophila* (formerly *Thiocapsa halophila*) was already found to be well-separated from representatives of the genus *Thiocapsa* (Guyoneaud *et al.*, 1998). DNA–DNA hybridization studies (Table 1) indicate that there is no close relationship between *Thiohalocapsa halophila* DSM 6210^T and the purple–red strain 5811^T or the type strain of the species *Thiocapsa roseopersicina*, with only 14 and 10 % DNA–DNA hybridization, respectively. These results confirm those of 16S rDNA sequence analysis, which have already led to the transfer of this halophilic species to a novel genus, *Thiohalocapsa* (Imhoff *et al.*, 1998). Concerning the relationship between the four purple–red *Thiocapsa* strains described in this study and the type species *Thiocapsa roseopersicina*, the results of DNA–DNA hybridization clearly separate the four purple–red strains from *Thiocapsa roseopersicina* (DNA relatedness values of 41–47 %). Moreover, the four purple–red strains formed a close cluster, with DNA similarities within the group of 70–87 %. On the basis of DNA–DNA hybridization studies, Brenner (1973) considered similarity values of 70 % and higher as representative of strains of the same species. Moreover, Johnson (1973) considered that different species from the same genus could have 20–60 % DNA similarity.

Table 2. Major properties of existing *Thiocapsa* species and *Thiocapsa marina* sp. nov.

Species: 1, *Thiocapsa marina*; 2, *Thiocapsa roseopersicina*; 3, *Thiocapsa pendens*; 4, *Thiocapsa rosea*; 5, *Thiocapsa litoralis*. Cells of all species are cocci and all species utilize sulfide, thiosulfate and acetate. ND, Not determined.

Characteristics	1	2	3	4	5
Cell diameter (μm)	1·5–3·0	1·2–3·0	1·5–2·0	2·0–3·0	1·5–2·5
Gas vesicles	−	−	+	+	−
Colour of cell suspensions	Purple–red	Rose-red	Rose-red	Rose-red	Rose-red
Major carotenoid*	Ok	Sp	Sp	Sp	Sp
DNA G+C content (mol%)	62·7–63·2	63·3–66·3	65·3	64·3	64·0
Vitamin requirement	−	−	B_{12}	B_{12}	B_{12}
Sulfate assimilation	−	+	−	−	+
pH optimum	7·5	7·3	6·7–7·5	6·7–7·5	6·5
NaCl optimum (%)	1–2	0	0	0	1
Substrates used:					
Hydrogen	+	+	−	−	ND
Butyrate	−	−	−	−	+
Fumarate	+	+	−	−	+
Succinate	+	+	−	−	+
Glycerol	+	+	−	−	−
Fructose	+	+	−	−	+
Glucose	−	−	−	−	+

*Ok, Okenone; Sp, spirilloxanthin.

On this basis, the four purple–red strains clearly belong to the genus *Thiocapsa* and form a separate species within this genus. Moreover, as stated by Stackebrandt & Goebel (1994), the presence of strong phenotypic coherency among strains should always be the deciding factor when grouping strains into one species. In the case of the four purple–red strains described in this paper, the presence of carotenoids of the okenone series, which is a feature of strong ecological relevance, together with the results of both 16S rDNA sequence analysis and DNA–DNA hybridization, suggest strongly that all four strains should be considered as members of a novel *Thiocapsa* species (Table 2). *Thiocapsa marina* sp. nov. is proposed as the name for this novel species and strain 5811^T (=DSM 5653^T=ATCC 43172^T) is the type strain.

Description of *Thiocapsa marina* sp. nov.

Thiocapsa marina (ma.ri′na. L. fem. adj. *marina* belonging to the marine environment).

Individual cells are spherical and 1·5–3·0 μm in diameter; cells occur singly or in pairs, tetrads or small aggregates. Division is by binary fission. Gram-negative. Non-motile. Cell suspension is purple–red. Photosynthetic membrane system is of the vesicular type. Contains bacteriochlorophyll *a* as photosynthetic pigment and okenone as main carotenoid. Phototrophic growth is observed under anoxic conditions in light; chemolithotrophic and chemoorganotrophic growth is seen under micro-oxic conditions in the dark. Electron donors for phototrophic growth are H_2, sulfide, elemental sulfur and thiosulfate. Globules of elemental sulfur are stored in cells as an intermediary product when grown photolithotrophically with reduced sulfur compounds. In the presence of sulfide and bicarbonate, the following compounds are photoassimilated: formate, acetate, propionate, lactate, pyruvate, malate, succinate, fumarate, glycerol, fructose, peptone and Casamino acids. Chemolithotrophic growth occurs with sulfide and thiosulfate; chemoorganotrophic growth occurs with acetate and pyruvate. Not capable of assimilatory sulfate reduction. No vitamins are required. Optimal pH for growth is 7·2–7·4 and optimal temperature is 30–35 °C. Grows in 0–8 % NaCl (optimum, 1–2 %). DNA G+C content of the type strain is 62·7 mol%.

The type strain is 5811^T (=DSM 5653^T=ATCC 43172^T), which was isolated from Prevost Lagoon on the French Mediterranean coast. Isolated from anoxic sediments and microbial mats exposed to light in coastal brackish to marine lagoons.

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