Thioclava pacifica gen. nov., sp. nov., a novel facultatively autotrophic, marine, sulfur-oxidizing bacterium from a near-shore sulfidic hydrothermal area

Dimitry Yu. Sorokin,^{1,2} Tatjana P. Tourova,¹ Elizaveta M. Spiridonova,³ Fred A. Rainey⁴ and Gerard Muyzer²

Correspondence

Dimitry Yu. Sorokin
soroc@inmi.host.ru or
D.Y.Sorokin@tnw.tudelft.nl

¹S. N. Winogradskii Institute of Microbiology RAS, Prospect 60-let Octyabrya 7/2, 117811 Moscow, Russia

²Department of Environmental Biotechnology, Delft University of Technology, 2628 BC Delft, The Netherlands

³Department of Microbiology, Faculty of Biology, Moscow State University, Moscow, Russia

⁴Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803-1715, USA

Strain TL 2^T was isolated on mineral medium with thiosulfate from a near-shore sulfidic hydrothermal area in Matupi Harbour on the island of New Britain, Papua New Guinea. The cells varied from long filaments with swollen ends, often aggregated, to short rods, depending on the growth conditions. The bacterium was obligately aerobic and grew autotrophically with thiosulfate as energy source or heterotrophically with organic acids and sugars. In thiosulfate-limited continuous culture, $\mu_{\max}$ and $Y_{\max}$ for autotrophic growth were 0.1 h⁻¹ and 3 g protein mol⁻¹, respectively. From the various reduced sulfur compounds tested, only thiosulfate and sulfide supported active respiration. Inorganic carbon was assimilated via the Calvin cycle. Presence of the 'green'-type of form I RubisCO gene was detected. Growth was possible from 15 to 47 °C with an optimum at 35 °C, pH 6.5–8.5 with an optimum at pH 8.0, and between 10 and 90 g NaCl l⁻¹ with an optimum at 35 g l⁻¹. Phylogenetic analysis based on 16S rRNA and *cbbL* gene sequences demonstrated that strain TL 2^T forms a separate lineage within the alpha-3 subdivision of the *Proteobacteria*, distantly related to the genera *Rhodovulum* and *Rhodobacter*. On the basis of these results, a novel genus and species, *Thioclava pacifica* gen. nov., sp. nov., is proposed to accommodate strain TL 2^T (=DSM 10166^T=UNIQEM 229^T).

INTRODUCTION

The ability to grow chemolithoautotrophically with sulfur compounds has been demonstrated in many facultative species that differ from classical thiobacilli (Kelly, 1989). Facultative species are especially interesting in their metabolic flexibility, allowing them to dominate in environments with low fluxes of both reduced inorganic and organic carbon compounds, insufficient to maintain substantial populations of highly specialized obligate autotrophs or

obligate heterotrophs (Beudecker *et al.*, 1982; Kuenen, 1989).

A near-shore sulfidic hydrothermal area in Matupi Harbour on New Britain (Papua New Guinea) is an example of an environment that can be beneficial for proliferation of facultatively autotrophic sulfur bacteria. There, a hot sulfide-containing volcanic seep mixes with cold sea water containing oxygen and organic compounds, originated from decaying shore vegetation (Ferguson & Lambert, 1972). However, the concentration of these compounds fluctuates significantly because of the active water dynamics, which do not favour domination of obligate species. Enumeration of different types of sulfur-oxidizing bacteria demonstrated the dominance of heterotrophic forms in most of the samples oxidizing thiosulfate either to sulfate or to tetrathionate (Sorokin, 1991). Among the tetrathionate-producing heterotrophs, a peculiar coccoid

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TL 2^T is AY656719 and the accession numbers for partial *cbbL* gene sequences of strain TL 2^T, *Rdv. sulfidophilum* DSM 1374^T, *Rdv. euryhalinum* DSM 4868^T and *Rdv. adriaticum* DSM 2781^T are AY656720–AY656723.

morphotype was dominant and was described as *Catenococcus thiocycli* (Sorokin, 1992; Sorokin *et al.*, 1996). Among the ten sulfate-forming heterotrophic strains, one isolate, strain TL 2^T, was able to grow autotrophically. The physiological properties and taxonomic position of this novel bacterium are described in this paper.

METHODS

The sampling point was situated above a hot sulfidic seep at 3 m depth, where the water temperature was 40 °C. The maximum sulfide concentration during low tide was up to 0.8 mM and the salinity was 30‰. The sulfur-oxidizing population from this sample was first enriched and then serially diluted on mineral medium with thiosulfate and sea water. The culture grown from the greatest dilution was streaked onto solid medium of the same composition and single colonies were isolated. When inoculated in a liquid mineral medium, these colonies always resulted in the growth of two types of bacteria. One type was represented by a *Thiomicrospira*-like, obligately autotrophic, sulfur-oxidizer unable to grow heterotrophically. Another type, represented by a filamentous heterotroph, was a minor component in mineral medium but dominated under purely organotrophic or mixotrophic conditions. Despite differences in metabolism, separation of the two organisms was difficult, because they grew in a stable association and even formed fused colonies. Finally, after eight to ten sequential passages on purely organic medium with acetate, the heterotrophic member was isolated in pure culture and designated strain TL 2^T.

The mineral medium for primary enrichment culture was prepared on the basis of filter-sterilized sea water supplemented with 10 mM thiosulfate and the pH-indicator bromothymol blue (from water-saturated solution, 1 ml l⁻¹). For further batch cultivation, an artificial medium was used containing (g l⁻¹): NaCl 25, K₂HPO₄ 0.5, NH₄Cl 0.5, MgSO₄·7H₂O 0.4, CaCl₂·2H₂O 0.1 and trace metal solution (Pfennig & Lippert, 1966) 1 ml l⁻¹. The last three components were added after sterilization. TL 2^T required biotin and thiamine for growth; pyridoxine had a growth-stimulating effect. Therefore, normally, a filter-sterilized mixture of these vitamins was supplied after medium sterilization to a final concentration of 50 µg l⁻¹ each. Thiosulfate was added after sterilization from 2 M stock solution to a final concentration of 10–20 mM. The initial pH was set at 7.5. Upon acidification, the pH of the medium was adjusted to 7.5 with a sterile 1 M NaHCO₃ solution. For heterotrophic and mixotrophic growth, the mineral base was supplemented with an organic carbon source (10–20 mM) and yeast extract (0.2 g l⁻¹) instead of the vitamin mixture. Autotrophic growth with hydrogen was tested on a basic mineral medium plus the vitamin mixture in a closed jar under a 1:1 mixture (v/v) of air/H₂. Growth under denitrifying conditions was tested using acetate or thiosulfate as electron donor and nitrate, nitrite or N₂O as electron acceptor. The medium was made anoxic by flushing with argon for 20 min. The methylotrophic potential of the isolate was examined with methanol (2 ml l⁻¹) or formate (20 mM) as substrates. For continuous cultivation, a 1.5 l laboratory fermenter with a 1 l working volume, fitted with pH and oxygen controls (Applikon), was used. The dissolved oxygen concentration was controlled at 50% air saturation by the stirring speed. The temperature was controlled at 30 °C. For chemical stability, the medium was supplied from two reservoirs, containing acidic and alkaline solutions in double strength. The acidic solution contained (l⁻¹) 1 g KH₂PO₄, 0.8 g NH₄Cl, 0.4 g MgCl₂·6H₂O, 0.2 g CaCl₂·2H₂O, 2 ml trace metal solution, 25 µl silicone antifoam and 10 ml HCl. The alkaline base included 50 g l⁻¹ NaCl and 2 g l⁻¹ of NaOH. Thiosulfate and acetate were sterilized separately as 2 M solutions and added to the alkaline base after sterilization. For

autotrophic growth, the final concentration of thiosulfate was 40 mM, and for mixotrophic conditions 10 mM acetate and 10–20 mM thiosulfate were used.

Respiratory activity of washed cells was measured in a 4 ml thermostatted cell with a Clark oxygen electrode (Yellow Spring Inc.) at 30 °C in 0.05 M potassium phosphate buffer, pH 7.5, supplemented with 25 g NaCl l⁻¹ and 0.2 g MgCl₂·6H₂O l⁻¹.

Cell-free extract was prepared by ultrasonic disruption of washed cell suspension (10–15 mg protein ml⁻¹) either in 0.05 M phosphate or Tris/HCl buffers at pH 7.0 and 8.0, respectively. RubisCO activity in cell-free extracts was measured according to Beudecker *et al.* (1980). Activities of sulfite-cytochrome *c* oxidoreductase and AMP-dependent sulfite dehydrogenase were determined in the cell-free extract as described by Kelly & Wood (1994). Qualitative tests for the presence of cytochrome oxidase and catalase were performed with single colonies from an acetate-grown culture using 1% TMPD (N,N,N',N'-tetramethyl-1,4-phenylenediamine dihydrochloride) and 3% H₂O₂, respectively. Thiosulfate and tetrathionate were analysed by cyanolysis (Kelly *et al.*, 1969), sulfide and sulfite colorimetrically according to Trüper & Schlegel (1964) and sulfate by HPLC (column ET 250/8/4 Nucleosil 10 Anion; 30 °C, carrier 0.025 M sodium salicylate, pH 4.0, flow rate 1.5 ml min⁻¹). Biomass protein was estimated by the Lowry method, after washing the pellet with a 2.5% NaCl solution twice to remove interfering sulfur compounds. Pigments were extracted with a methanol/acetone mixture (3:7, v/v) at 4 °C for 12 h and the extract was analysed spectrophotometrically. For electron microscopy, cells were pre-fixed with glutaraldehyde (2.5% v/v) and then post-fixed with 1% OsO₄ + 2% NaCl (w/v), dehydrated and embedded into resin. Thin sections were stained with uranyl acetate and lead citrate.

Total DNA extraction, amplification and sequencing of 16S rRNA and of *cbbL* genes were performed as described previously (Sorokin *et al.*, 2003; Spiridonova *et al.*, 2004). Phylogenetic analyses based on nucleotide sequences of 16S rRNA genes and putative amino acid sequences deduced from *cbbL* sequences were performed using different treeing algorithms realized in the TREECON (Van de Peer & De Wachter, 1994) and PHYLIP (Felsenstein, 1989) software packages.

RESULTS

Morphology

Cell morphology of strain TL 2^T was highly variable, depending on the growth conditions. In diluted organic media, it grew as long non-motile filaments with swollen ends, often aggregated into tufts or rosettes (Fig. 1a–c). On rich organic media and in autotrophic chemostat culture, small uniform rods dominated, some cells being motile by means of a polar flagellum. The cell wall had a structure typical of Gram-negative bacteria. Autotrophically grown cells differed from cells grown under heterotrophic conditions by possessing an extended periplasm (Fig. 1d). Carboxysome-like structures were not observed in the cells grown autotrophically. Colonies of TL 2^T on organic media were up to 10 mm in diameter, flat, convex and pinkish; old colonies turned yellowish. On thiosulfate mineral agar, colonies were much smaller (1–2 mm in diameter), colourless and without sulfur deposition.

Strain TL 2^T is a mesophilic and neutrophilic bacterium, growing at temperatures from 15 to 47 °C (optimum 35 °C)

Fig. 1. Cell morphology of strain TL 2. (a–c) Phase-contrast microphotographs of TL 2^T growing in filamentous aggregates on medium with 5 mM acetate and 0.1 g l⁻¹ of yeast extract. (d) Electron micrograph of thin section of TL 2^T cells with extended periplasm (P) and polar polyphosphate granules (Pp) from autotrophic thiosulfate-limited chemostat culture; N, nucleoid. Bars, 10 µm (a–c) and 0.5 µm (d).

and at pH 6.5–8.5 (optimum pH 7.5–7.8). The bacterium was NaCl-dependent with the NaCl range suitable for growth between 10 and 80 (optimum 30–40) g l⁻¹. It was oxidase- and catalase-positive. No specific light-absorbing pigments were detected in the methanol/acetone extract of TL 2^T cells between 300 and 1000 nm.

Metabolism

TL 2^T grew chemoautotrophically with thiosulfate as an electron donor and heterotrophically with acetate, succinate, malate, lactate, pyruvate, propionate, butyrate, glycolate, glyoxylate, malonate, ethanol, glycerol, mannitol, D-glucose, D-maltose, sucrose, D-cellobiose, proline, lysine, alanine, glutamate, glutamine, aspartate and asparagine. Growth was not observed with methanol, formate, methylamine, benzoate, D-ribose, D-fructose, D-galactose, D-melibiose, starch, casein, Tween 80, aromatic amino acids, leucine, arginine, threonine or serine. It was unable to ferment glucose or to grow anaerobically under denitrifying conditions either autotrophically or heterotrophically. Ammonium and nitrate, but not nitrite, urea or dinitrogen served as nitrogen sources.

In batch cultures with acetate, thiosulfate was oxidized mostly to sulfate during the exponential phase of growth (Fig. 2). Significant amounts of sulfite were detected in the medium as an intermediate of thiosulfate oxidation. To obtain a stable autotrophic culture of TL 2^T on mineral medium with thiosulfate as energy source, two or three adaptive transfers from the mixotrophic and five or six transfers from the heterotrophic media were necessary. Up to 5 mM sulfite was transiently produced in the course of thiosulfate consumption. The $\mu_{\max}$ and $Y_{\max}$ values in the activated autotrophic culture were 0.06 h⁻¹ and 1.6–1.7 g

protein (mol thiosulfate)⁻¹, respectively. In continuous mixotrophic (acetate + thiosulfate) culture with 10 mM of each substrate, sulfite was not accumulated in the effluent at a dilution rate (D) up to 0.05 h⁻¹ but, when the influent thiosulfate concentration was increased up to 20 mM, sulfite started to accumulate at $D > 0.04$ h⁻¹. The autotrophic continuous culture with 40 mM thiosulfate started from the mixotrophic culture after 2 weeks adaptation period at low D (0.03 h⁻¹) reached $\mu = 0.1$ h⁻¹, after which wash-out of the culture was observed. In contrast to batch cultures, no sulfite production was found in the autotrophic continuous culture and the molar growth yield was about

Fig. 2. Growth and oxidation of thiosulfate in batch mixotrophic culture (acetate + thiosulfate, 20 mM each) of strain TL 2^T. ●, Biomass (mg protein l⁻¹); ○, thiosulfate (mM); ▲, sulfate (mM); △, sulfite (mM).

Table 1. Parameters of growth and activity of strain TL 2^T

Growth conditions						Respiration rate [nmol O ₂ (mg protein) ⁻¹ min ⁻¹]		RubisCO activity [nmol CO ₂ (mg protein) ⁻¹ min ⁻¹]
Culture mode	Acetate (mM)	S ₂ O ₃ ²⁻ (mM)	D (h ⁻¹)	Y ₁ *	Y ₂ *	S ₂ O ₃ ²⁻	HS ⁻	
Batch	20	0	0.18	ND	8.34	48	45	0
	0	20	0.06	ND	1.72	240	260	45.2
Continuous	10	20	0.03	—	—	320	400	5.4
	0	40	0.03	1.30	1.75	380	440	ND
	0	40	0.05	1.43	2.08	650	690	ND
	0	40	0.10	2.08	2.63	1010	1120	34.5

ND, Not determined.

*Specific growth yield expressed as mg C_{org} (mmol S₂O₃²⁻)⁻¹ (Y₁) or mg protein (mmol S₂O₃²⁻)⁻¹ (Y₂); —, not possible to assign biomass to either substrate.

2-fold higher (Table 1). One of the possible reasons for such differences might be more stable pH conditions in the continuous culture.

RubisCO activity was undetectable in TL 2^T cells grown heterotrophically. It was very low, but detectable, in mixotrophically grown cells and increased significantly during autotrophic growth (Table 1). Oxygen-consumption experiments with washed cells demonstrated that TL 2^T can oxidize thiosulfate and sulfide at almost equal rates, and activity was present in heterotrophically grown cells at a very low level and increased by two orders of magnitude in the autotrophic continuous culture (Table 1). The affinity constant for these substrates was very high (4–7 µM). Elemental sulfur and tetrathionate did not stimulate oxygen consumption. The ability of TL 2^T to oxidize sulfite was of special interest because of its transient accumulation in batch cultures. Our experiments demonstrated that sulfite started to accumulate in those cultures where the thiosulfate loading exceeded the oxidizing capacity of the bacterium and when the culture was grown under inappropriate conditions (e.g. in the absence of vitamins or at an unsuitable pH). Hence, it might be speculated that sulfite was not a natural intermediate of thiosulfate oxidation in TL 2^T. This was supported by the fact that washed cells were not capable of active sulfite oxidation (3–5 nmol mg protein⁻¹ min⁻¹) and did show sulfite dehydrogenase activity at a detectable level.

Phylogenetic analysis

The G+C content in the DNA of TL 2^T was 63.1 ± 0.3 mol%. Phylogenetic analysis based on the almost complete 16S rRNA gene sequence of TL 2^T placed the novel isolate into the α-3 group of the *Proteobacteria* within the order '*Rhodobacterales*'. In the phylogenetic tree constructed with the neighbour-joining algorithm, strain TL 2^T formed a separate branch located between the purple non-sulfur bacteria of the genera *Rhodobacter* and *Rhodovulum* (Fig. 3). However, a low value of bootstrap support (24 %) showed an uncertain position for its branch point. The

phylogenetic trees constructed either by maximum-parsimony or maximum-likelihood algorithms had the same topologies (data not shown). The nucleotide sequence similarity was almost the same with different species of the genera *Rhodobacter* and *Rhodovulum*, ranging from 92.4 to 95.0 %. There was no close relatedness between the strain TL 2^T and the recently described non-photosynthetic members of the *Rhodobacter*–*Rhodovulum* clade, *Albidovulum inexpectatum* (Albuquerque *et al.*, 2002) and *Pseudorhodobacter ferrugineus* (Uchino *et al.*, 2002), which had 92.8 and 92.5 % sequence similarity to strain TL 2^T, respectively.

Fig. 3. Phylogenetic position of strain TL 2^T within the α-3 group of the *Proteobacteria* based on 16S rRNA gene sequence analysis. The tree was reconstructed from evolutionary distances by using the neighbour-joining method. The scale bar represents five inferred nucleotide changes per 100 nucleotides. The percentage of bootstraps was derived from 100 resamplings; values greater than 80 were considered as significant.

To obtain more information on the phylogenetic position of the novel bacterium among the *Rhodobacter*–*Rhodovulum* clade, an additional phylogenetic analysis of the translated sequence of the *cbbL* gene (encoding the large RubisCO subunit) was performed. Previous work indicated that most of the species of the genus *Rhodobacter* (*Rhodobacter capsulatus*, *Rba. blasticus* and *Rba. veldkampii*) contained the ‘green’-type of form I RubisCO, *Rhodobacter azotoformans* contained both ‘green’ and ‘red’ types and *Rhodobacter sphaeroides* contained only ‘red’-type (Paoli *et al.*, 1998; Uchino & Yokota, 2003). Using our previously designed primer set for the two types of *cbbL* gene (Spiridonova *et al.*, 2004), the ‘green’ type of form I RubisCO was detected in strain TL 2^T and the type strains of three species of the genus *Rhodovulum* (*Rhodovulum sulfidophilum*, *Rdv. adriaticum* and *Rdv. euryhalinum*). Approximately 750-bp *cbbL* gene fragments were amplified and sequenced from the DNA of these strains. The identity values between the translated amino acid *cbbL* sequences of strain TL 2^T, new *cbbL* sequences of *Rhodovulum* species and analogous sequences of *Rhodobacter* species available from GenBank ranged from 82.6 to 86.5 %; the identity values to analogous sequences of other autotrophic bacteria ranged from 75.0 to 85.3 %. These values are reflected in the branching pattern of the corresponding *cbbL* tree constructed with the maximum-likelihood-treeing algorithm (Fig. 4). Although the *cbbL* gene sequence of the TL 2^T is more closely affiliated with the coherent *cbbL* sequence subcluster of the alphaproteobacterial *Rhodobacter*–*Rhodovulum* clade and the betaproteobacterium *Hydrogenophilus thermoluteolus* within the ‘green’-type sequence cluster (bootstrap value of 76 % for the common branching point), it formed a novel lineage only distantly related to *cbbL* of this subcluster. The topology of the *cbbL* tree supported conclusions on the isolated

position of the novel isolate among its photosynthetic neighbours within the *Rhodobacter*–*Rhodovulum* clade.

DISCUSSION

The novel bacterium strain TL 2^T represents a relatively rare type of marine, facultatively autotrophic, sulfur-oxidizing bacterium. For unknown reasons, this type of organism is difficult to isolate from marine habitats, even using a chemostat technique (Gottschal & Kuenen, 1980) successfully applied for the enrichment of facultative species from freshwater samples. In the case of TL 2^T, the presence of a small number of the cells with peculiar morphology in a uniform enrichment culture of obligately autotrophic, sulfur-oxidizing, *Thiomicrospira*-like bacteria indicated the possible development of an interesting phenotype. Close association of the facultatively autotrophic TL 2^T with an obligate autotroph resembles the well-known association between *Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*) and the facultatively autotrophic alphaproteobacterium *Acidiphilium acidophilum* (formerly *Thiobacillus acidophilus*) (Guay & Silver, 1975), which also grew in fused colonies where the latter consumed organic excretion products of the autotroph.

Phylogenetic analysis placed the novel bacterium in the α -subclass of the *Proteobacteria*. This subclass is remarkable in the strong representation of lithoheterotrophic and facultatively autotrophic sulfur-oxidizing species. Moreover, some of the aerobic sulfur-oxidizing alphaproteobacteria still express non-functional bacteriochlorophyll *a* or contain its gene (Yurkov & Beatty, 1998). This might indicate direct descent from a common ancestral anaerobic, non-sulfur, purple bacterium. The facultatively

Fig. 4. Phylogenetic tree based on the analysis of the form I RubisCO showing position of strain TL 2^T among autotrophic bacteria. The tree, based on amino acid sequences deduced from *cbbL* nucleotide sequences, was reconstructed by the maximum-likelihood method. The scale bar represents ten substitutions per 100 amino acid sites. Percentages of bootstraps were derived from 1000 resamplings; values greater than 70 were considered as significant. Numbers [1] and [2] indicate the presence of two different genes in the same organism. Accession numbers in parentheses refer to the nucleotide sequences; numbers in bold represent sequences determined in this study. Note that GenBank accession number U55037 erroneously gives the strain as *Hydrogenophaga pseudoflava* ‘DSM 1083’ (Y. M. Kim, personal communication).

autotrophic strain TL 2^T and the thiosulfate-oxidizing heterotroph *Albidovulum inexpectatum* (Albuquerque *et al.*, 2002) are phylogenetically even more closely related to anaerobic phototrophs than to chemotrophic representatives of the α -*Proteobacteria*. But, apart of the ability to oxidize thiosulfate and similarity of their habitats (marine thermal waters), strain TL 2^T and *Albidovulum inexpectatum* differed significantly in phenotypes, such as autotrophy, temperature and salt range. One of the interesting questions remaining to be answered is why this particular subclass, in contrast, for example, to the β - or γ -subclasses of the *Proteobacteria*, is almost completely lacking in obligate autotrophs.

Finally, regarding the unique phylogenetic position and distinct phenotype, this isolate is proposed to represent a novel genus and species with the name *Thioclava pacifica* gen. nov., sp. nov.

Description of *Thioclava* gen. nov.

Thioclava (Thi.o.cla'va. Gr. neut. n. *thion* sulfur; L. fem. n. *clava* stick, staff, cudgel, club; N.L. fem. n. *Thioclava* sulfur-oxidizing swollen rod).

Gram-negative rods of varying size from long filaments with swollen ends, clustered in aggregates, to single small rods, rarely motile by one polar flagellum. Obligately aerobic and facultatively autotrophic, sulfur-oxidizing bacteria. Grow chemoautotrophically with thiosulfate and heterotrophically with simple organic compounds. Unable to utilize hydrogen as electron donor and to grow methylotrophically. Inorganic carbon is assimilated via the Calvin cycle. Contain the 'green form' of type I RubisCO. UQ-10 is the only respiratory lipoquinone present. The type species of the genus is *Thioclava pacifica*.

Description of *Thioclava pacifica* sp. nov.

Thioclava pacifica (pa.ci'fi.ca. L. fem. adj. *pacifica* pacific, peacemaking, and, by extension, pertaining to the Pacific Ocean, where the type strain was isolated).

The description is identical to that of the genus, with the following additions. The following substrates are utilized for heterotrophic growth: acetate, succinate, malate, lactate, pyruvate, propionate, butyrate, glycolate, glyoxylate, malonate, ethanol, glycerol, mannitol, D-glucose, D-maltose, sucrose, D-cellobiose, proline, lysine, alanine, glutamate, glutamine, aspartate and asparagine. No growth on methanol, formate, methylamine, benzoate, D-ribose, D-fructose, D-galactose, D-melibiose, starch, casein, Tween 80, aromatic amino acids, leucine, arginine, threonine or serine. Unable to ferment glucose or to grow anaerobically under denitrifying conditions either autotrophically or heterotrophically. Ammonium salts and nitrate serve as nitrogen sources. Thiamine and biotin are required for growth. Oxidase- and catalase-positive. Grows within a pH range from 6.5 to 8.5 (optimum 7.5–7.8), temperature range from 15 to 47 °C (optimum 35 °C) and NaCl range from 10

to 80 g l⁻¹ (optimum 30–40 g l⁻¹). The G+C content of the DNA of the only strain known in the species is 63.1 ± 0.3 mol%.

The type strain, TL 2^T (=DSM 10166^T = UNIQEM 229^T), originated from sulfidic thermal sea water in Matupi Harbour, New Britain, Papua New Guinea.

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REFERENCES

- Albuquerque, L., Santos, J., Travassos, P., Nobre, M. F., Rainey, F. A., Wait, R., Empadinhas, N., Silva, M. T. & da Costa, M. S. (2002). *Albidovulum inexpectatum* gen. nov., sp. nov., a nonphotosynthetic and slightly thermophilic bacterium from a marine hot spring that is very closely related to members of the photosynthetic genus *Rhodovulum*. *Appl Environ Microbiol* **68**, 4266–4273.
- Beudecker, R. F., Cannon, G. C., Kuenen, J. G. & Shively, J. M. (1980). Relations between D-ribulose-1,5-bisphosphate carboxylase, carboxysomes and CO₂-fixing capacity in the obligate chemolithotroph *Thiobacillus neapolitanus* grown under different limitations in the chemostat. *Arch Microbiol* **124**, 185–189.
- Beudecker, R. F., Gottschal, J. G. & Kuenen, J. G. (1982). Reactivity versus flexibility in thiobacilli. *Antonie van Leeuwenhoek* **48**, 39–51.
- Felsenstein, J. (1989). PHYLIP - Phylogenetic Inference Package version 3.2. *Cladistics* **5**, 164–166.
- Ferguson, J. & Lambert, I. B. (1972). Volcanic exhalations and metal enrichments at Matupi Harbour, New Britain, T.P.N.G. *Econ Geol* **67**, 25–37.
- Gottschal, J. C. & Kuenen, J. G. (1980). Selective enrichment of facultatively chemolithotrophic thiobacilli and related organisms in the chemostat. *FEMS Microbiol Lett* **7**, 241–247.
- Guay, R. & Silver, M. (1975). *Thiobacillus acidophilus* sp. nov.; isolation and some physiological characteristics. *Can J Microbiol* **21**, 281–288.
- Kelly, D. P. (1989). Physiology and biochemistry of unicellular sulfur bacteria. In *Autotrophic Bacteria*, pp.193–217. Edited by H. G. Schlegel & B. Bowien. Berlin: Springer.
- Kelly, D. P. & Wood, A. P. (1994). Enzymes involved in micro-biological oxidation of thiosulfate and polythionates. *Methods Enzymol* **243**, 501–520.
- Kelly, D. P., Chambers, L. A. & Trudinger, P. A. (1969). Cyanolysis and spectrophotometric estimation of trithionate in mixture with thiosulfate and tetrathionate. *Anal Chem* **41**, 898–901.
- Kuenen, J. G. (1989). Comparative ecophysiology of the nonphoto-trophic sulfide-oxidizing bacteria. In *Microbial Mats: Physiological Ecology of Benthic Microbial Communities*, pp. 349–365. Edited by I. Cohen & E. Rosenberg. Washington, DC: American Society for Microbiology.
- Paoli, G. C., Soyer, F., Shively, J. & Tabita, F. R. (1998). *Rhodobacter capsulatus* genes encoding form I ribulose-1,5-bisphosphate carboxylase/oxygenase (*cbbLS*) and neighbouring genes were acquired by a horizontal gene transfer. *Microbiology* **144**, 219–227.

- Pfennig, N. & Lippert, K. D. (1966).** Über das Vitamin B₁₂ – bedürfnis phototropher Schwefel bacterien. *Arch Microbiol* **55**, 245–256 (in German).
- Sorokin, D. Yu. (1991).** Oxidation of reduced sulfur compounds in volcanically active regions of the Plenty Bay (New Zealand) and Matupi Harbour (New Britain, Papua New Guinea). *Izv Akad Nauk S S R Ser Biol*, 376–387 (in Russian).
- Sorokin, D. Yu. (1992).** *Catenococcus thiocyclus* gen. nov., sp. nov. – a new facultatively anaerobic bacterium from a near-shore sulphidic hydrothermal area. *J Gen Microbiol* **138**, 2287–2292.
- Sorokin, D. Y., Robertson, L. A. & Kuenen, J. G. (1996).** Sulfur cycling in *Catenococcus thiocyclus*. *FEMS Microbiol Ecol* **19**, 117–126.
- Sorokin, D. Yu., Tourova, T. P., Sjollem, K. A. & Kuenen, G. J. (2003).** *Thialkalicoccus nitratreducens* sp. nov., a nitrate-reducing member of an autotrophic denitrifying consortium from a soda lake. *Int J Syst Evol Microbiol* **53**, 1779–1783.
- Spiridonova, E. M., Berg, I. A., Kolganova, T. V., Ivanovsky, R. N., Kuznetsov, B. B. & Tourova, T. P. (2004).** A system of oligonucleotide primers for the amplification of the ribulose-1,5-bisphosphate carboxylase/oxygenase genes of different taxonomic groups of bacteria. *Mikrobiologiya* **73**, 316–325 (in Russian).
- Trüper, H. G. & Schlegel, H. G. (1964).** Sulphur metabolism in *Thiorhodaceae*. 1. Quantitative measurements on growing cells of *Chromatium okenii*. *Antonie van Leeuwenhoek* **30**, 225–238.
- Uchino, Y. & Yokota, A. (2003).** “Green-like” and “red-like” RuBisCO *cbbL* genes in *Rhodobacter azotoformans*. *Mol Biol Evol* **20**, 821–830.
- Uchino, Y., Hamada, T. & Yokota, A. (2002).** Proposal of *Pseudorhodobacter ferrugineus* gen. nov., comb. nov., for a non-photosynthetic marine bacterium, *Agrobacterium ferrugineum*, related to the genus *Rhodobacter*. *J Gen Appl Microbiol* **48**, 309–319.
- Van de Peer, Y. & De Wachter, R. (1994).** TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl Biosci* **10**, 569–570.
- Yurkov, V. V. & Beatty, T. (1998).** Aerobic anoxygenic phototrophic bacteria. *Microbiol Mol Biol Rev* **62**, 695–724.