



Microorganisms of the oxidative and reductive part of the sulphur cycle at a shallow-water hydrothermal vent in the Aegean Sea (Milos, Greece)

Jan KUEVER^{1*}, Stefan M. SIEVERT², Heike STEVENS³,
Thorsten BRINKHOFF³, and Gerard MUYZER⁴

(1) *Department of Microbiology, Max-Planck-Institute for Marine Microbiology, Celsiusstr.1, D-28359 Bremen, Germany*

** Correspondence to: Dr. Jan Kuever, E-mail: jkuever@mpi-bremen.de*

(2) *present address: Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA*

(3) *present address: Institute for the Chemistry and Biology of the Marine Environment (ICBM), University of Oldenburg, P.O. Box 2503, D-26111 Oldenburg, Germany*

(4) *present address: Kluyver Laboratory for Biotechnology, Dept. of Microbiology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands*

Introduction

At a solitary gaseous hydrothermal vent located at a water depth of 8 m in Palaeochori Bay, Milos (Greece) sediment cores were taken by SCUBA divers along a transect from the center to the surrounding area. Sediment cores were subsampled by slicing and used for cultivation-based and molecular analyses of the microbial community. Because of the continuous supply of sulphide at this site in combination with overlaying oxygen-containing seawater chemolithoautotrophic microorganisms have an ideal habitat. As a result of incomplete microbial sulphide oxidation, sulphur accumulated above the sediment and may represent either an electron donor or an electron acceptor for reductive processes. In contrast to deep-sea vents this shallow-water system is influenced by the availability of organic material produced by phototrophic organisms (sea grass, diatoms and cyanobacteria) resulting in high abundances of heterotrophic microorganisms. Nevertheless, the continuous release of sulphide and sulphur acting as electron donor and their oxidation to sulphur and sulphate indicates a high turnover of sulphur compounds. Therefore our study focused on microorganisms that are involved in the oxidative and reductive part of the sulphur cycle.

Material and methods

Sampling and slicing of sediment cores were performed as indicated by Sievert et al. (1999). Sediment cores were taken at 30, 60, 110 and 200 cm from the vent center and subsampled at 1 cm intervals up to a depth of 6 cm. Bacteria

were isolated from serial dilutions (Most Probable Number Counts; three parallel tubes) using different specific media. Aerobic chemolithoautotrophic sulphur-oxidizing bacteria (SOB) were cultivated and isolated according to Sievert & Kuever (2000). The same medium, prepared under anoxic conditions with nitrate as terminal electron acceptor, was used for cultivation and isolation of chemolithoautotrophic, nitrate-reducing SOB. Sulphate-reducing bacteria were cultivated and isolated in artificial seawater medium (Widdel & Bak, 1992) containing lactate (20 mM) or acetate (20 mM) as electron donors. For the cultivation of sulphur-reducing bacteria, 20 mM acetate was used as electron donor and elemental sulphur (added aseptically from a sterile slurry of sulphur flower in distilled water) or polysulphide (from a sterile stock solution) were used as electron acceptors in the same medium without sulphate. Sulphur reducing bacteria were only obtained from the upper part of the sediment (0-1 cm) where the in situ temperature was 23-45 °C. At deeper depths the temperature could increase up to 88 °C and no sulphur-reducing bacteria were found. Only at a distance of 200 cm from the center of the vent, where the temperature was below 30 °C even at 20 cm depth, sulphur-reducing bacteria were isolated from a depth between 6 to 8 cm. Isolates were obtained by repeated application of agar dilution series using fumarate (20 mM) as electron donor and acceptor. Fumarate was used as substrate because it can be fermented by most mesophilic sulphur-reducing bacteria and will not cause a precipitation like sulphur in solid media. All cultures were incubated at their approximate in situ temperature (23 to 45 °C). Best growth of pure cultures was between 28 and 35 °C.

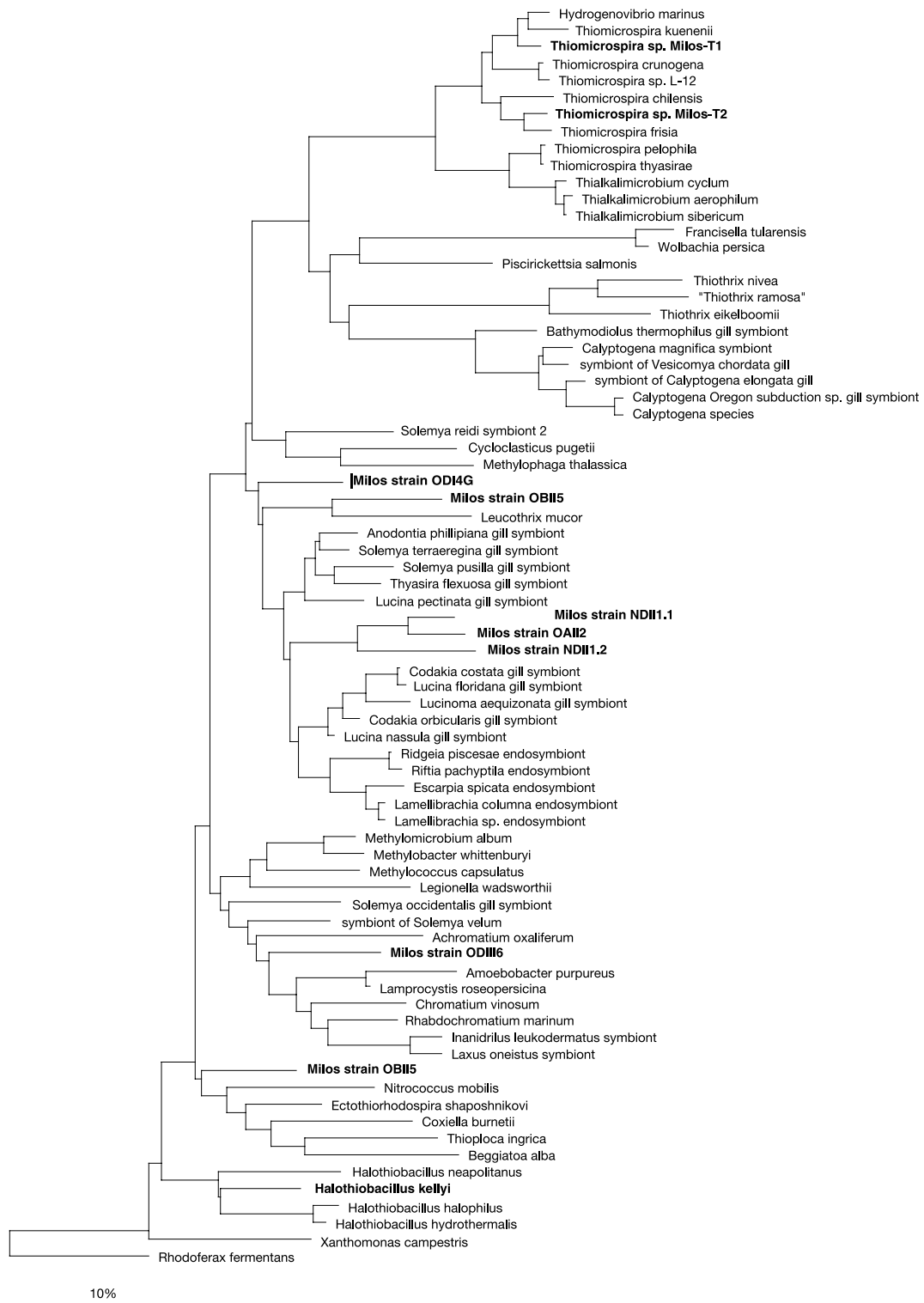


Figure 1. Phylogenetic tree showing the affiliations of 16S rDNA sequences from sulphur-oxidizing bacteria obtained from Milos to selected reference sequences of the gamma-subdivision of the *Proteobacteria*. The tree was calculated by Maximum-Likelihood analysis and corrected with a termini filter and a filter which considered only 50% conserved regions of the 16S rRNA of gamma-Proteobacteria. The sequence of *Rhodoferrax fermentans*, beta-subdivision of the *Proteobacteria*, was used as out group. The bar represents 10% estimated sequence divergence. Milos strain NDII.1, NDII.2, and OAI2 can also use nitrate as electron acceptor.

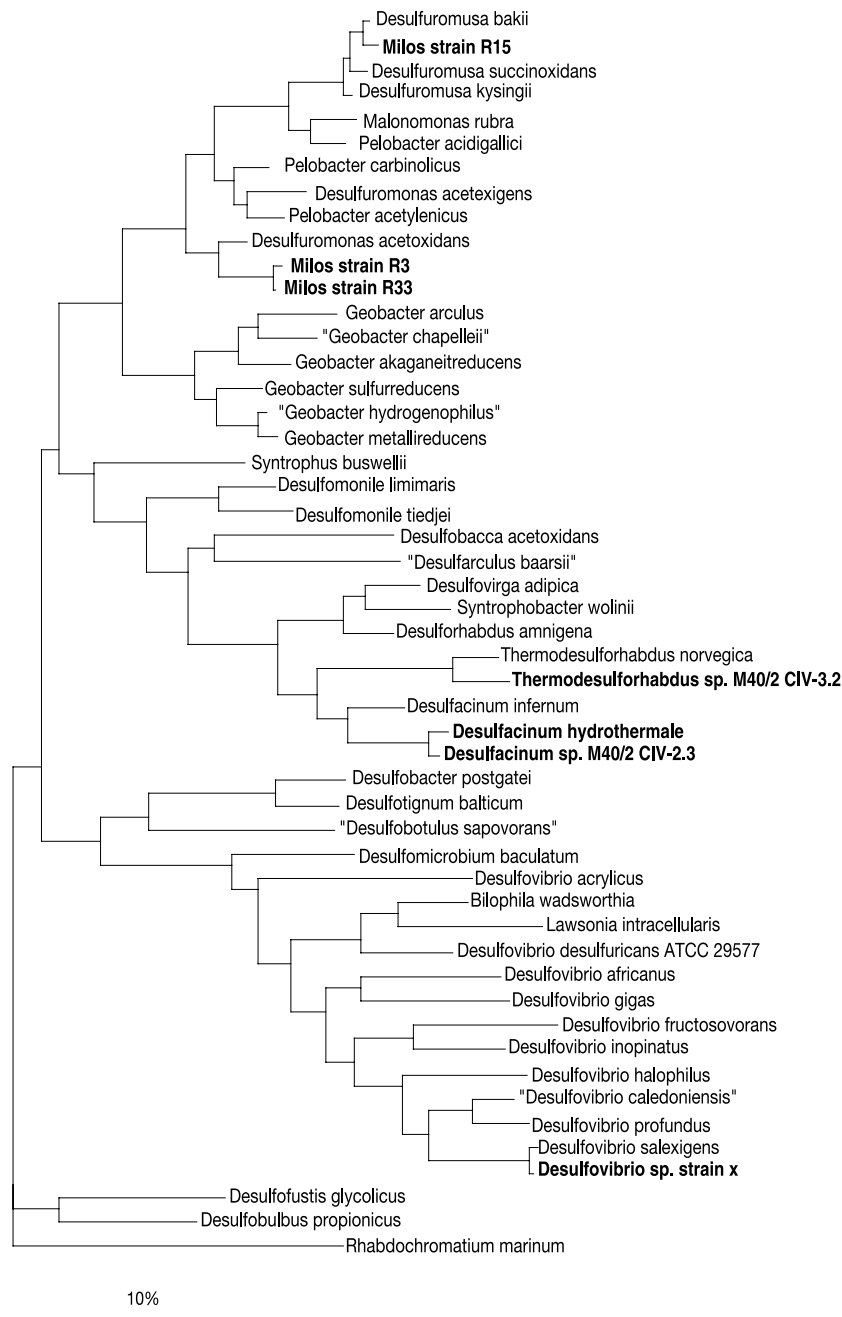


Figure 2. Phylogenetic tree showing the affiliations of 16S rDNA sequences from the sulphur- and sulphate-reducing bacteria obtained from Milos to selected reference sequences of the delta-subdivision of the *Proteobacteria*. The tree was calculated by Maximum-Likelihood analysis and corrected with a termini filter and a filter which considered only 50% conserved regions of the 16S rRNA of delta-Proteobacteria. The sequence of *Rhabdochromatium marinum*, gamma-subdivision of the *Proteobacteria* (Dilling et al., 1996) was used as out group. The bar represents 10% estimated sequence divergence.

PCR amplifications and sequencing of the 16S rRNA genes of all isolates obtained were performed using standard general primers as described previously (Sievert & Kuever, 2000). The sequences were loaded into the 16S rRNA sequence data base of the Technical University Munich using the program package ARB (Strunk et al., <http://www.mikro.biologie.tu-muenchen.de>). The tool ARB_

ALIGN was used for sequence alignment. The alignment was visually inspected and corrected manually. Tree topologies were evaluated by performing maximum parsimony, neighbor joining, and maximum likelihood analysis. Only sequences with at least 1400 nucleotides were used for the calculation of different trees.

Results and Discussion

Sulphur-oxidizing bacteria

All chemolithoautotrophic SOB obtained as isolates were mesophilic members of the gamma-subdivision of the *Proteobacteria*, a group containing most marine SOB. The highest numbers for aerobic sulphur oxidizing bacteria (1.42×10^6 cells gww^{-1}) were found at 200 cm from the vent center at a depth of 1-2 cm accounting for 0.41% of the total cell numbers. DGGE analysis and phylogenetic analysis of isolates obtained from the highest dilutions showed a clear dominance of *Thiomicrospira* spp. within this habitat (Sievert et al., 1999; Brinkhoff et al., 1999). Two different *Thiomicrospira* spp. were isolated in pure culture, strain Milos T1 and strain Milos T2. Other SOB like *Halothiobacillus kellyi* Sievert et al. 2000, which was isolated from a low dilution (Sievert et al., 2000) were never detected by molecular analysis. *Halothiobacillus kellyi* shows a higher acid tolerance than the *Thiomicrospira* spp. and might represent a typical isolate obtained by an enrichment. Nevertheless we were also able to isolate several new aerobic and nitrate-reducing SOB which never exceeded 10^3 cells gww^{-1} . In contrast to *Thiomicrospira* and *Halothiobacillus*, they show a different phylogenetic position within the gamma-subdivision of the Proteobacteria (see Fig.1). Some of them seemed to be closely affiliated to endo- and ectosymbiotic SOB, a unique feature among all culturable SOB. Thermophilic SOB were not detected.

Sulphur- and sulphate-reducing bacteria

All sulphur-reducing bacteria isolated from the vent site (strain R3, strain R15, strain R33) were mesophilic members of the genera *Desulfuromonas* and *Desulfuromusa* within the family *Desulfuromonaceae* (see Fig. 2). Their physiological properties were similar to already described species. The predominant sulphate-reducing bacteria isolated from the vent site were thermophilic and belonged to the genera *Thermodesulfurhabdus* and *Desulfacinum* within the family *Syntrophobacteraceae* (Sievert & Kuever, 2000) (see Fig. 2). They were able to use a large variety of different electron donors for dissimilatory sulphate reduction and could grow chemolithoautotrophically with H_2 and CO_2 . These organisms seem to be well adapted to the elevated temperature which is increasing with depth and gradients of the vent site. Only one mesophilic sulphate-reducing bacterium (*Desulfovibrio* sp. strain x) was obtained which showed a very high similarity to *Desulfovibrio salexigens* Postgate & Campbell, 1966 (see Fig. 2). Interestingly this strain could also use fructose as electron donor.

Conclusions

The presence of only mesophilic SOB indicates that ideal growth conditions for these chemolithoautotrophic microor-

ganisms occur where sulphide and cold sea water meet, resulting in a moderate temperature regime. All sulphur-reducing bacteria were also mesophiles and restricted to certain electron donors. This can be explained by the availability of sulphur and suitable electron donors at the vent system which both might originate from SOB. In contrast, sulphate-reducing bacteria which can utilize a large variety of different electron donors or show chemolithoautotrophic growth were only found deeper in the sediment at elevated temperatures; even there sulphate would not be depleted. A detailed description of the new isolates and their physiological properties will be published soon.

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