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Marine acidophilic sulfur-oxidizing bacterium requiring salts for the oxidation of reduced inorganic sulfur compounds

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Abstract An acidophilic sulfur-oxidizing bacterium was isolated from seawater, and designated as strain SH. Strain SH was a Gram-negative, rod-shaped and motile bacterium, which had an optimum temperature and pH value for growth of 30°C and 4.0, respectively. The mol% guanine plus cytosine of the DNA was 46.0. Chemolithotrophic growth was observed with elemental sulfur and tetrathionate at pH 4.0, and was not observed with ferrous ion. The isolate was able to utilize carbon dioxide as a carbon source, and was unable to grow heterotrophically with yeast extract or glucose. The growth of strain SH was activated in medium supplemented with NaCl. However, LiCl and KCl did not sustain the growth of strain SH. The results indicate that strain SH was an acidophilic, halophilic, and obligately chemolithotrophic sulfur-oxidizing bacterium. Phylogenetic analysis based on 16S rDNA sequences indicated that strain SH had a close relationship to *Acidithiobacillus thiooxidans*. The oxidizing activities of sulfur and sulfite with resting cells were stimulated not only by the addition of NaCl, but also by KCl and LiCl. The oxidation of sulfite was inhibited by ionophores, carbonyl cyanide-*m*-chlorophenylhydrazone (CCCP), and monensin, and respiratory inhibitors, KCN and 2-heptyl-4-hydroxy-quinoline-N-oxode (HQNO).

Keywords *Acidithiobacillus* · Acidophile · Chemolithotroph · Halophile · Phylogeny · Sulfur-oxidizing bacterium

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Introduction

The problem of how to detoxify sediments and soils contaminated with heavy metals and toxic organic chemicals is one that is currently receiving much attention worldwide. A variety of chemolithotrophic and heterotrophic bacteria are responsible for the mobilization of metals from sulfide ores in the bioleaching process. Although *Acidithiobacillus ferrooxidans* (an iron and sulfur oxidizer) and *Acidithiobacillus thiooxidans* (a sulfur oxidizer) were commonly thought to be important bacteria for the bioleaching process, *Acidithiobacillus caldus* (a moderately thermophilic sulfur oxidizer) and *Leptospirillum ferrooxidans* (an iron oxidizer) have recently been shown to play an important role in the metal-mobilizing process (Rawlings et al. 1999). Although *A. thiooxidans* and *A. caldus* are acidophilic sulfur-oxidizing bacteria which were detected in the bioleaching process, almost all these bacteria cannot grow in media supplemented with high concentrations of NaCl. An obligately chemolithotrophic and acidophilic aerobe, *Thiobacillus prosperus*, which uses both elemental sulfur and ferrous ion as energy sources and is tolerant to NaCl, was isolated from a marine geothermal field; it is thought to be useful for bioleaching processes in salt-containing environments (Huber and Stetter 1989). Recently, we have isolated an acidophilic sulfur- and iron-oxidizing bacterium which requires NaCl for growth (Kamimura et al. 2001a). Although there have been many reports on NaCl-tolerant or NaCl-requiring sulfur-oxidizing bacteria, such as *Halothiobacillus hydrothermalis* (Durand et al. 1993), *Halothiobacillus halophilus* (Wood and Kelly 1991), *Halothiobacillus neapolitanus* (Kelly and Harrison 1989), *Halothiobacillus kellyi* (Sievert et al. 2000), and *Thiomicrospira* sp. (Kuenen and Veldkamp 1972; Ruby et al. 1981; Ruby and Jannasch 1982; Jannasch et al. 1985), these bacteria were not acidophiles. Acidophilic sulfur-oxidizing bacteria that are tolerant to NaCl or require NaCl for growth may be useful in developing remedial technologies for salt-containing environments

contaminated with heavy metals. In this report, we describe some properties of an NaCl-requiring and acidophilic sulfur-oxidizing bacterium from the marine environment.

Materials and methods

Microorganisms and isolation of bacteria

A. thiooxidans ON106 was used as a terrestrial strain in this study. The cells were statically grown in sulfur medium (pH3.0) containing (w/v): sulfur (1%), $(NH_4)_2SO_4$ (0.3%), K_2HPO_4 (0.05%), $MgSO_4{\cdot}7H_2O$ (0.05%), KCl (0.01%), and $Ca(NO_3)_2$ (0.001%). The pH of the medium was adjusted with 1 N sulfuric acid. The medium supplemented with 2% NaCl was used for the growth of marine strains. The sulfur-oxidizing bacterium was isolated by using an enrichment culture and a single-colony isolation technique. Seawater samples and sediments collected from Seto Inland Sea, Japan, were added to sulfur medium supplemented with 2% NaCl, and incubated at 30°C. When the growth of bacteria was detected in the medium, a sample of culture medium was spread on gellan gum plates supplemented with 0.2% tetrathionate as a substrate and incubated at 30 °C. Colonies appeared on the plate and were isolated by single colony isolation procedure which was repeated three times. The final isolate (strain SH) was preserved in sulfur medium (pH 4.0) supplemented with 2% NaCl.

Growth experiments

Cell growth was monitored by counting cell numbers with a microscope. Cells were statically grown in sulfur medium (pH 4.0) in a flask and were separated from sulfur particles by filtering the culture through filter paper (Toyo paper filter No. 5B). The number of cells in the filtrate was counted with a hemacytometer after dilution with 0.1 N sulfuric acid, when necessary. The optimal pH value for growth of the isolate was determined by using medium adjusted to various initial pH values. The initial pH value was maintained by adding 1 N Na_2CO_3.

Measurement of carbon dioxide uptake

The activity of carbon dioxide fixation was measured by the amount of Na_2CO_3 incorporated into the resting cells. The reaction mixture consisted of 4.0 ml of 0.1 M β-alanine-SO_4^{2-} buffer (pH 4.0), washed resting cells (1 mg of protein), 1 μmol carrier Na_2CO_3, 1 μCi $Na_2{}^{14}CO_3$, 0.175 M NaCl, and 53 mg of elemental sulfur in a total reaction mixture of 5 ml. The reaction was started by adding $Na_2{}^{14}CO_3$, carrier Na_2CO_3, and elemental sulfur. After incubation at 30°C, the reaction was stopped by adding 0.5 ml of 20 mM mercuric chloride. The reaction mixture was passed through a 0.22-μm membrane filter. The filter was completely solubilized into the counting solution (Scintisol EX-H). The radioactivity was measured with an Aloka LSC635 liquid scintillation counter.

Measurement of oxidation of reduced sulfur compounds

The oxidation of elemental sulfur, sulfite, thiosulfate, and sulfide was measured by the amount of oxygen uptake due to the oxidation of the sulfur compounds using a Biological Oxygen Monitor (Yellow Spring Instrument, Ohio, USA). Reaction mixtures contained: washed resting cells (0.5–1 mg of protein), 0.1 M β-alanine-SO_4^{2-} buffer (pH 4.0), and 32 mg of elemental sulfur, 0.33 mM sulfite, 20 μM thiosulfate, or 20 μM sulfide in the total volume of 3 ml. The reaction was started by adding each sulfur compound to the reaction mixture.

PCR amplification, determination of 16S rDNA, and phylogenetic analysis

Strain SH was phylogenetically analyzed on the basis of 16S rDNA sequenced by the method described previously (Kamimura et al. 2001b). Genomic DNA was extracted by phenol-chloroform after lysis by lysozyme, and 16S rDNA fragment was amplified by PCR with nondegenerated primers corresponding to positions 8–27 (forward) and 1,539–1,515 (reverse) in the *Escherichia coli* 16S rDNA sequence. The amplification was performed in a thermal cycler using an initial denaturation step at 94°C for 2 min followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 60°C for 90 s and extension at 72°C for 90 s. The final cycle included a 4-min extension step at 72°C. The 16S rDNA amplified by PCR was purified using a Geneclean kit (Funakoshi, Tokyo) and then directly sequenced with an automated sequence analyzer (model DSQ-100L; Shimadzu, Kyoto). The 16S rDNA sequence of strain SH was aligned to the prealigned sequences with secondary structures provided by the Ribosomal Database Project, University of Illinois (Maidak et al. 1999). The phylogenetic tree was inferred and established by using the PHYLIP program, version 3.75c. The DNADIST program was used to calculate evolutionary distances. The phylogenetic tree was constructed from evolutionary distance data using the program NEIGHBOR. One hundred bootstrap replicate resampling data sets for DNADIST were generated with the program SEQBOOT, to provide confidence estimates for tree topologies. The nucleotide sequence data of strain SH reported in this paper appeared in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB080298.

Other methods

An electron microscopic observation of negatively stained cells was carried out with a Hitachi H-7510 electron microscope with an accelerating voltage at 80 kV. DNA base composition of strain SH was measured by reverse-phase high-pressure liquid chromatography after the DNA had been enzymatically hydrolyzed to free nucleotides (Tamaoka and Komagata 1984). Protein was determined by the Lowry method with crystalline bovine serum albumin as the reference protein (Lowry et al. 1951).

Results

Isolation, morphology and physiological characteristics of sulfur-oxidizing bacterium strain SH

A single colony was isolated from the solid plate inoculated with an enrichment culture of the seawater sample, which had been collected from Seto Inland Sea, Japan. One isolate, which had the highest growth yield in sulfur medium supplemented with 0.35 M (2%) NaCl, was selected, designated as strain SH, and used for further experiments. Strain SH was a Gram-negative, rod-shaped (0.3–0.4 μm in width and 0.8–1.3 μm in length), and motile bacterium having a single polar flagellum. The optimum growth with elemental sulfur was observed at pH 4.0. Slight growth was observed at pH 1.0, and no growth was observed above pH 6.0. The optimum temperature for growth with elemental sulfur at pH 4.0 was 30°C, with no growth above 37°C or below 20°C. The mean guanine plus cytosine (G+C) content of the DNA from strain SH was 46.0 mol%. Chemolithotrophic growth of strain SH was observed in medium supplemented with elemental sulfur or tetrathionate as a substrate. The generation time obtained at

30°C in medium (pH 4.0) supplemented with elemental sulfur or tetrathionate was 30.4 h and 23.9 h, respectively. Although conclusions about the possibility of growth with thiosulfate, sulfide, or sulfite were not obtained, strain SH could oxidize these compounds. Sulfide-, thiosulfate-, and sulfite-oxidizing activities were 1.95, 1.14, and 2.23 μl O_2 /min/mg of protein, respectively. Growth was not activated in sulfur medium supplemented with yeast extract (0.05%) or glucose (0.2%). Growth was not also observed in basal salt medium supplemented with Fe^{2+} or Fe^{2+} and yeast extract (0.02%). The activity of carbon dioxide fixation was measured with resting cells of strain SH grown in sulfur medium supplemented with NaCl. The cells fixed 7.87 nmol carbon dioxide into 1 mg of protein after 60 min of incubation when elemental sulfur was used as an energy source. The growth was activated in sulfur medium supplemented with NaCl and Na_2SO_4, but not in sulfur medium supplemented with KCl and LiCl. Although the growth of all terrestrial *A. thiooxidans* strains tested was inhibited by increasing the concentration of NaCl, as shown with *A. thiooxidans* strain ON106 in Fig. 1, strain SH had an optimal concentration of NaCl for the growth at 0.35 M, as shown in Fig. 1. The culture medium used for growth experiments contained 4.2 mM KCl. It is suggested that acidophiles use a K^+/H^+ antiporter to regulate internal pH instead of a Na^+/H^+ antiporter (Matin 1999); thus, strain SH was cultured in medium prepared without K^+ and supplemented with 2% NaCl to examine the effect of K^+ on the growth of strain SH. Growth of strain SH was inhibited in the medium prepared without K^+. Based on the characteristics described above, strain SH was characterized as an acidophilic and obligately chemolithotrophic sulfur-oxidizing bacterium which required salts for growth.

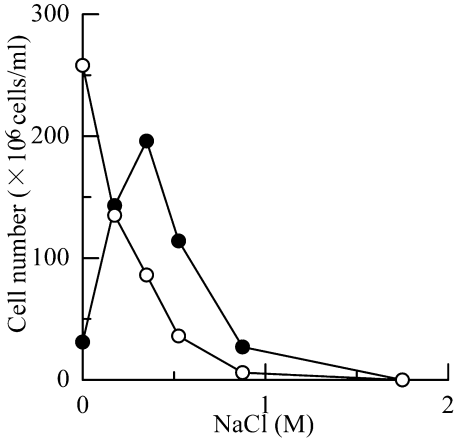

Fig. 1 Growth of strain SH and *Acidithiobacillus thiooxidans* strain ON106 in medium supplemented with various concentrations of NaCl. ● growth of strain SH, ○ growth of *A. thiooxidans* strain ON106

Phylogenetic analysis of sulfur-oxidizing bacterium

Strain SH was phylogenetically analyzed based on 16S rDNA sequence. An almost complete 16S rDNA sequence (1,482 bp) of strain SH was determined by sequencing the PCR-amplified 16S rDNA fragment. The result of phylogenetic analysis based on the sequence data is shown in Fig. 2. Strain SH was placed in the gamma subdivision of the *Proteobacteria* because of its relationship with *A. thiooxidans* and *A. ferrooxidans* (Sievert et al. 2000). The highest level of sequence similarity for strain SH was the level of sequence similarity for *A. thiooxidans* (99.4%, obtained from the sequence alignment used to infer the phylogenetic tree).

Effect of NaCl on the oxidation of reduced sulfur compounds

As strain SH required NaCl for its growth, the effect of NaCl on the oxidation of reduced sulfur compounds was examined. Sulfur-oxidizing activity was detected in a broad pH range without a clear optimum. The effect of NaCl was examined at pH 4.0, at which pH strain SH grew maximally. Although sulfur-oxidizing activity in

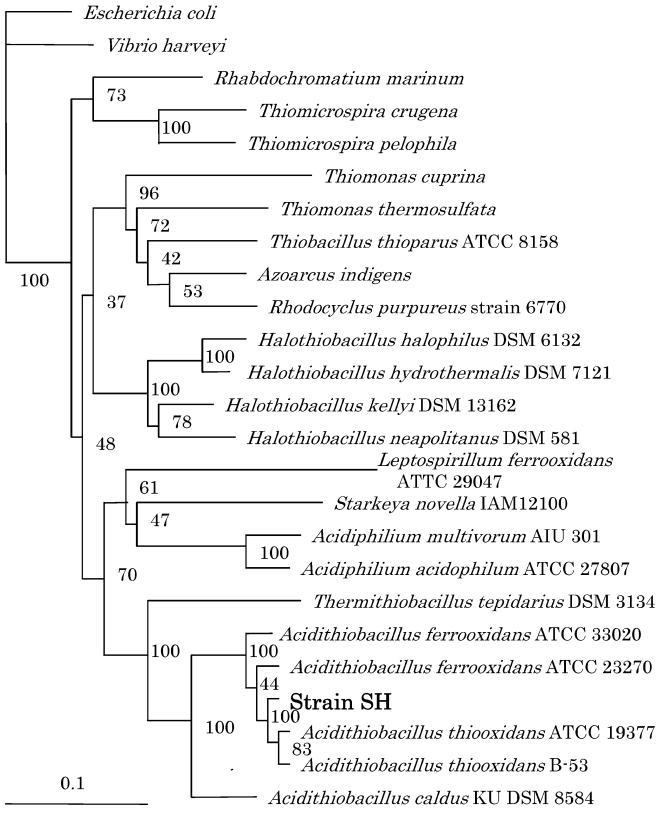

Fig. 2 Phylogenetic relationship among iron- and sulfur-oxidizing bacteria and position of strain SH on the basis of 16S rDNA sequence. The *numbers* on the branches are bootstrap values obtained for 100 replicates (neighbor joining). The *scale bar* represents a 10% estimated sequence divergence

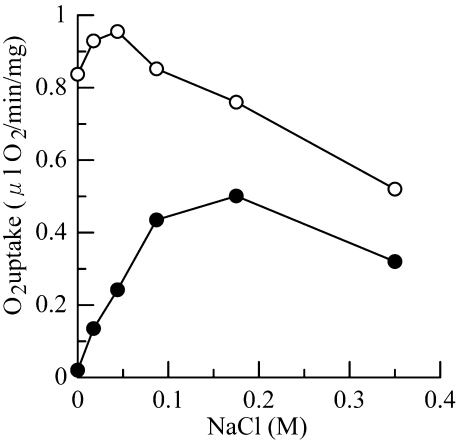

Fig. 3 Effect of NaCl on the oxidation of sulfur in strain SH (●) and *A. thiooxidans* strain ON106 (○)

terrestrial *A. thiooxidans* strain ON106 was slightly stimulated at 45 mM NaCl and inhibited with increasing concentration of NaCl, the activity in strain SH was very low in the reaction mixture without NaCl and highly stimulated in the reaction mixture supplemented with 0.175 M NaCl as shown in Fig. 3. The stimulation was not specific for NaCl and was also observed with 0.175 M Na_2SO_4, LiCl or KCl (data not shown). Although *A. thiooxidans* strain ON106 still oxidized sulfur at a grater rate than did strain SH at 0.35 M NaCl, which was the optimal concentration for the growth of strain SH, growth of strain ON106 was inhibited at this concentration, as shown in Fig. 1. In most sulfur-oxidizing bacteria, elemental sulfur is oxidized via sulfite as an intermediate to sulfate. Therefore, sulfite-oxidizing activity was measured with resting cells. The activity was detected in strain SH and had an optimum pH at 4.0. The sulfite-oxidizing activity was also stimulated in the reaction mixture supplemented with NaCl. The activity was stimulated about 4.5 times by 0.175 M NaCl compared with the activity without NaCl, and also stimulated about 2.5, 3.5, and 5 times by 175 mM LiCl, KCl, and 87.5 mM Na_2SO_4, respectively.

Effect of uncouplers and inhibitors on the oxidation of sulfite

CCCP and 2,4-dinitrophenol (DNP) are known as uncouplers or protonophores. These agents affected the membrane potential of *Acidiphilium acidophilum* (formerly *Thiobacillus acidophilus*) grown with glucose as a growth substrate (Matin et al. 1982). Monensin is known as a sodium ionophore. Concentrations of CCCP, DNP, and monensin, giving 50% inhibition of sulfite oxidation, were 3, 10, and 5 μM, respectively. The effects of respiratory inhibitors on the activity of sulfur and sulfite oxidation were examined. Although concentrations of KCN and HQNO giving 50% inhibition of sulfur oxidation were 80 and 5 μM, respectively, concentrations giving 50% inhibition of sulfite oxidation were 1 μM KCN and 80 nM HQNO, respectively.

Discussion

An acidophilic and halotolerant metal-mobilizing bacterium, *Thiobacillus prosperus*, has already been isolated from a marine geothermal field (Huber and Stetter 1989). We have also isolated an iron- and sulfur-oxidizing bacterium from the marine environment (Kamimura 2001a). These bacteria can oxidize both sulfur compounds and ferrous ion as energy sources. Strain SH is a slightly halophilic and acidophilic bacterium which utilizes only sulfur compounds as energy sources, but cannot utilize ferrous ion. Although strain SH had a close relationship with an acidophilic sulfur-oxidizing bacterium, *A. thiooxidans*, in the phylogenetic analysis based on 16S rDNA sequences, *A. thiooxidans* with halophilic characteristics has not yet been reported. In addition, although the mean G+C content of DNA from *A. thiooxidans* is reported to be 51–53 mol% (Holt et al. 1994), the content (46 mol%) of strain SH was slightly lower than that of *A. thiooxidans*. The results suggested that strain SH was a novel strain within *Acidithiobacillus*, although more detailed examination, such as DNA–DNA hybridization between *A. thiooxidans* and strain SH, should be carried out.

Seawater is usually strongly buffered by $NaHCO_3$ on the alkaline side of neutrality. Although strain SH cannot grow at the alkaline pH of regular seawater, an acidophilic iron- and sulfur-oxidizing bacterium has already been isolated from Seto Inland Sea (Kamimura 2001a), from where the samples used in this report were collected. Although *Thiobacillus prosperus* was isolated from a marine geothermal field, there is no information about underwater volcanic activity in Seto Inland Sea. As acidophilic iron- and sulfur-oxidizing bacteria can be isolated from nonacidic soils and freshwater samples, it is thought that there may be acidic microenvironments suitable for their growth in such environments. The isolation of strain SH from a natural seawater sample suggested that there may also be acidic microenvironments in the sampling area and strain SH may play an important role in sulfur cycling in such environments.

Although the growth of strain SH was stimulated by sodium ions, and not by KCl and LiCl, a small amount of K^+ seemed to be necessary for the growth of strain SH because its growth in medium prepared with no K^+ and supplemented with 2% NaCl was inhibited. As it is suggested that acidophiles use a K^+/H^+ antiporter to regulate internal pH, this result suggests that such functions may be involved in strain SH cells. As strain SH required a relatively high concentration of sodium ions for growth, the effect of sodium ions on the oxidation of sulfur and sulfite was investigated because involvement of Na^+-translocating respiratory components has been reported in sodium-requiring marine

heterotrophic bacteria (Ventosa et al. 1998; Unemoto and Hayashi 1993). However, sulfur and sulfite oxidation were stimulated not only by sodium ions, but also by KCl and LiCl. As KCl and LiCl did not sustain the growth of strain SH, growth-specific processes, which specifically require sodium ions, rather than sulfur compound oxidation must be involved in strain SH. The effects of sodium ions on growth-specific processes are now under investigation.

The results from experiments using respiratory inhibitors suggested that a respiratory quinone might be involved in the oxidation of sulfite, because HQNO strongly inhibited sulfite-oxidizing activity at very low concentration (80 nM). Although sulfite-oxidizing activity measured by oxygen uptake did not specifically require sodium ions, components that specifically require sodium ions may be involved in the system. The determination of respiratory components involved in sulfite oxidation in strain SH is now in progress.

It is well known that a sodium motive force is used to drive energy-requiring membrane-bound processes, such as the transport of substrates and the rotation of the flagellum in marine bacteria. The sulfite oxidation was inhibited by the protonophore, CCCP, and the sodium ionophore, monensin. The results suggested that sulfite oxidation occurred in the cytoplasm of strain SH, and both proton and sodium motive forces might be used for the incorporation of sulfite into the cytoplasm. Although the clear evidence for the requirement of sodium motive force in the rotation of the flagellum of strain SH has not yet been obtained, it would be very interesting to know how the sodium motive force is generated in strain SH. Thus, strain SH may be a useful bacterium for the investigation of sodium-requiring processes in an acidophilic chemolithotrophic bacterium, and may also be used to develop remedial technologies for salt-containing environments contaminated with heavy metals.

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References

Durand P, Reysenbach AL, Prieur D, Pace N (1993) Isolation and characterization of *Thiobacillus hydrothermalis* sp. nov., a mesophilic obligately chemolithotrophic bacterium isolated from a deep-sea hydrothermal vent in Fijji Basin. Arch Microbiol 159:36–44

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994) Bergey's manual of determinative bacteriology. Williams and Wilkins, Baltimore, MD

Huber H, Stetter KO (1989) *Thiobacillus prosperus* sp. nov., represents a new group of halotolerant metal-mobilizing bacteria isolated from a marine geothermal field. Arch Microbiol 151:479–485

Jannasch HW, Wirsen CO, Nelson DC, Robertson LA (1985) *Thiomicrospira crunogena* sp. nov., a colorless sulfur-oxidizing bacterium from a deep-sea hydrothermal vent. Int J Syst Bacteriol 35:422–424

Kamimura K, Kunomura K, Wakai S, Murakami K, Sugio T (2001a) Some properties of a novel obligately autotrophic iron-oxidizing bacterium isolated from seawater. Hydrometallurgy 59:373–381

Kamimura K, Wakai S, Sugio T (2001b) Identification of *Thiobacillus ferrooxidans* trains based on restriction fragment length polymorphism analysis of 16S rDNA. Microbios 105: 141–152

Kelly DP, Harrison AP (1989) Genus *Thiobacillus* Beijerink. In: Staley JT, Bryant MP, Pfenning N, Holt JG (eds) Bergey's manual of systematic bacteriology, 1st edn, vol. 3. Williams and Wilkins, Baltimore, MD, pp 1842–1858

Kuenen JG, Veldkamp H (1972) *Thiomicrospira pelophila*, gen. n., sp. n., a new obligately chemolithotrophic colorless sulfur bacterium. Antonie Van Leeuwenhoek 38:241–256

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurements with the Folin phenol reagents. J Biol Chem 193:265–275

Maidak BL, Cole JR, Parker CT Jr, Garrity GM, Larsen N, Li B, Lilburn TG, McCaughey MJ, Olsen GJ, Overbeek R, Pramanik S, Schmidt TM, Tiedge JM, Woese CR (1999) A new version of RDP (Ribosomal Database Project). Nucleic Acids Res 27:171–173

Matin A (1999) pH homeostasis in acidophiles. Novartis Foundation Symposium 1999 221:152–163

Matin A, Wilson B, Zychlinsky E, Matin M (1982) Proton motive force and the physiology basis of delta pH maintenance in *Thiobacillus acidophilus*. J Bacteriol 150:582–591

Rawlings DE, Tributsch H, Hansford GS (1999) Reasons why '*Leptospirillum*'-like species rather than *Thiobacillus ferrooxidans* are the dominant iron-oxidizing bacteria in many commercial processes for the biooxidation of pyrite and related ores. Microbiology 145:5–13

Ruby EG, Jannasch HW (1982) Physiological characteristics of *Thiomicrospira* sp. strain L-12 isolated from deep-sea hydrothermal vents. J Bacteriol 149:161–165

Ruby EG, Wirsen CO, Jannasch HW (1981) Chemolithoautotrophic sulfur-oxidizing bacteria from the Galapagos Rift hydrothermal vents. Appl Environ Microbiol 42:317–322

Sievert SM, Heidorn T, Kuever J (2000) *Halothiobacillus kellyi* sp. nov., a mesophilic, obligately chemolithoautotrophic, sulfur-oxidizing bacterium isolated from a shallow-water hydrothermal vent on the Aegean Sea, and emended description of the genus *Halothiobacillus*. Int J Syst Evol Microbiol 50:1229–1237

Tamaoka J, Komagata K (1984) Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microbiol Lett 25:125–128

Unemoto T, Hayashi M (1993) Na^+-translocating NADH-quinone reductase of marine and halophilic bacteria. J Bioenerg Biomembr 25:385–391

Ventosa A, Nieto J, Oren A (1998) Biology of moderately halophilic aerobic bacteria. Microbiol Mol Biol Rev 62:504–544

Wood AP, Kelly DP (1991) Isolation and characterization of *Thiobacillus halophilus* sp. nov., a sulphur-oxidizing autotrophic eubacterium from a Western Australian hypersaline lake. Arch Microbiol 150:277–280