Reclassification of 'Thiobacillus prosperus' Huber and Stetter 1989 as Acidihalobacter prosperus gen. nov., sp. nov., a member of the family Ectothiorhodospiraceae

Juan Pablo Cárdenas,[1,2,3] Rodrigo Ortiz,[1,3] Paul R. Norris,[4] Elizabeth Watkin[5] and David S. Holmes[1,2]

Correspondence
David S. Holmes
dsholmes2000@yahoo.com

[1]Departamento de Ciencias Biologicas, Facultad de Ciencias Biologicas, Universidad Andres Bello, Santiago, Chile

[2]Center for Bioinformatics and Genome Biology, Fundacion Ciencia & Vida, Santiago, Chile

[3]Laboratorio de Ecofisiología Microbiana, Fundacion Ciencia & Vida, Santiago, Chile

[4]Environment and Sustainability Institute, University of Exeter, UK

[5]School of Biomedical Sciences, Curtin University, Perth, Australia

Analysis of phylogenomic metrics of a recently released draft genome sequence of the halotolerant, acidophile 'Thiobacillus prosperus' DSM 5130 indicates that it is not a member of the genus *Thiobacillus* within the class *Betaproteobacteria* as originally proposed. Based on data from 16S rRNA gene phylogeny, and analyses of multiprotein phylogeny and average nucleotide identity (ANI), we show that it belongs to a new genus within the family *Ectothiorhodospiraceae*, for which we propose the name *Acidihalobacter* gen. nov. In accordance, it is proposed that 'Thiobacillus prosperus' DSM 5130 be named *Acidihalobacter prosperus* gen. nov., sp. nov. DSM 5130[T] (=JCM 30709[T]) and that it becomes the type strain of the type species of this genus.

'*Thiobacillus prosperus*' DSM 5130, is a halotolerant (growth with up to 0.6 M NaCl) acidophile (<pH 3) that was isolated from a geothermally heated seafloor at Porto di Levante, Vulcano, Italy (Huber & Stetter, 1989). It was originally classified into the genus *Thiobacillus* as '*Thiobacillus prosperus*' on the basis of its phenotype and other classical criteria (sulfur-oxidizing, autotrophic, Gram-stain-negative rods and with DNA G+C content of 64 mol%) (Huber & Stetter, 1989). However, the genus name was potentially confusing because '*Thiobacillus prosperus*' exhibited insignificant DNA hybridization to members of the genus *Thiobacillus* such as *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, *Thiobacillus neapolitanus* and *Thiobacillus thioparus* (Huber & Stetter, 1989). *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* were later reclassified as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*, respectively (Kelly & Wood,

2000) and the genus *Acidithiobacillus* was moved out of the class *Gammaproteobacteria* into the class *Acidithiobacillia* (Williams & Kelly, 2013).

Later studies, based on 16S rRNA phylogeny, suggested that '*Thiobacillus prosperus*' was a member of the class *Gammaproteobacteria*, with species of the family *Ectothiorhodospiraceae* as near relatives (Simmons & Norris, 2002). During a recent study of the classification of *Acidiferrobacter thiooxydans*, a 16S rRNA-based phylogeny suggested a position for '*Thiobacillus prosperus*' as a member of the family *Ectothiorhodospiraceae* of the class *Gammaproteobacteria* (Hallberg *et al.*, 2011). In addition, in a note in *Bergey's Manual of Systematic Bacteriology* (Kelly *et al.*, 2005), it was proposed that '*Thiobacillus prosperus*' be removed from the genus *Thiobacillus*, because of its close phylogenetic resemblance (>95 % 16S rRNA gene sequence identity) with '*Acidihalobacter aeolicus*' (strain V6=DSM 14174) and to '*Acidihalobacter ferrooxidans*' (strain V8=DSM 14175). However, despite its suggestive relationship with the family *Ectothiorhodospiraceae*, '*Thiobacillus prosperus*' remains to be formally classified.

Recently, the draft genome sequence of '*Thiobacillus prosperus*' was released (Ossandon *et al.*, 2014) providing

Abbreviations: ANI, average nucleotide identity; COG, clusters of orthologous groups.

The GenBank/EMBL/DDBJ accession number for the genome sequence of strain DSM 5130 is JQSG00000000.

Three supplementary tables and two supplementary figures are available with the online Supplementary Material.

an opportunity to re-evaluate its taxonomical position using phylogenomic strategies (Delsuc *et al.*, 2005).

A multi-locus phylogenomic tree was reconstructed, based on a multiple alignment of concatenated sequences of 50 ribosomal protein families retrieved using COG (clusters of orthologous groups) classification from 95 proteobacteria (Tatusov *et al.*, 2003). A list of the NCBI accession numbers for the genomes, including their taxonomical data, and a list of the COG families used in the elaboration of the phylogenomic tree are provided, respectively, in Tables S1 and S2 (available in the online Supplementary Material). The alignment of the concatenated sequences was made using MAFFT (Katoh & Standley, 2014). A phylogenomic tree was reconstructed using the maximum-likelihood method on PhyML version 3 using the LG model (Guindon *et al.*, 2010); the latter was defined as the most appropriate model for the data using Model-Generator software (Keane *et al.*, 2006). The phylogenomic tree was reconstructed taking into account discrete estimated gamma values and amino acid frequencies (+G +F), and the tree topology was optimized using a combination of the best results of nearest-neighbor interchange (NNI) and subtree pruning and regrafting (SPR) strategies (Guindon *et al.*, 2010).

Average nucleotide identity (ANI) analysis was carried out using the ANI calculator (http://enve-omics.ce.gatech.edu/ani/) (Goris *et al.*, 2007). A list of ANI values obtained from a comparison of the '*Thiobacillus prosperus*' genome against other sequenced members of the family *Ectothiorhodospiraceae* is provided in Table S3.

A 16S rRNA sequence-based phylogenetic tree of organisms from the family *Ectothiorhodospiraceae* was reconstructed using sequences obtained from the prokaryotic 16S rRNA database of NCBI-BLAST and aligned in MAFFT (Katoh & Standley, 2014). The phylogenetic tree was inferred using the maximum-likelihood method based on the Tamura–Nei model, defined as the most appropriate model by ModelGenerator (Keane *et al.*, 2006). Tree reconstruction included the use of discrete Gamma distribution and allowing some sites to be evolutionarily invariable (G+I+).

A multi-locus phylogenomic tree was reconstructed from a multiple alignment of concatenated sequences of 50 ribosomal protein families from 95 proteobacterial organisms including '*Thiobacillus prosperus*' (Yutin *et al.*, 2012). The resulting full phylogenomic tree is shown in Fig. S1. The region of the tree that includes '*Thiobacillus prosperus*' has been expanded for clarity (Fig. 1). '*Thiobacillus prosperus*' is positioned within the family *Ectothiorhodospiraceae* in the order *Chromatiales* of the class *Gammaproteobacteria* (Figs 1 and S1). This is consistent with its placement based on 16S rRNA gene phylogenetic analysis (Fig S2) and with a previously described 16S rRNA gene phylogenetic analysis (Hallberg *et al.*, 2011).

Within the family *Ectothiorhodospiraceae*, '*Thiobacillus prosperus*' has the closest phylogenomic similarity with the genus *Ectothiorhodospira* (Fig. 1). The genomic DNA of '*Thiobacillus prosperus*' exhibits 64.5 mol% G+C content (Ossandon *et al.*, 2014), which is in agreement with the range of 50.5–69.7 mol% DNA G+C for members of the family *Ectothiorhodospiraceae* (Imhoff, 2005). Also, consistent with the placement of '*Thiobacillus prosperus*' close to the genus *Ectothiorhodospira* is the requirement for chloride for growth exhibited by both these groups (Davis-Belmar *et al.*, 2008; Imhoff, 2005).
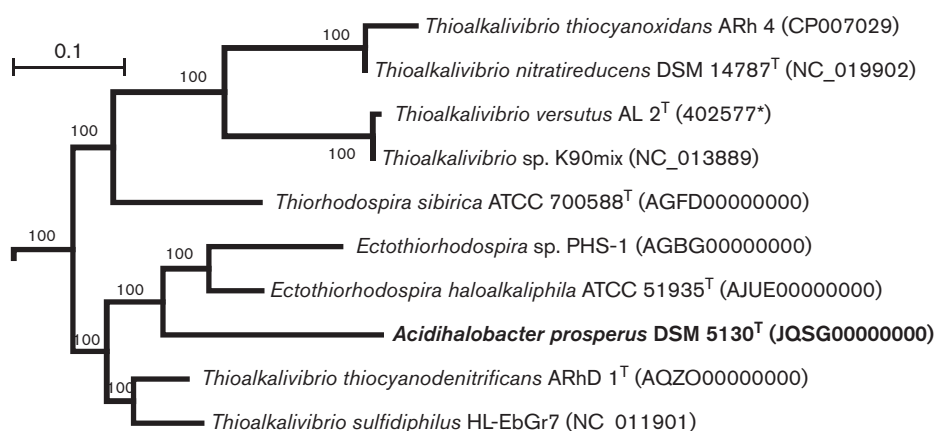
Fig. 1. Phylogenetic position of *Acidihalobacter prosperus* gen. nov., sp. nov. (formerly '*Thiobacillus prosperus*') in the family *Ectothiorhodospiraceae* within the order *Chromatiales* of the class *Gammaproteobacteria* based upon multi-locus concatenation of 50 ribosomal proteins. Results of the approximate likelihood ratio test returning χ^2-based parametric branch support values are shown. Genome accession numbers for each organism are shown. The list of COGs used for tree elaboration is given in Table S2. The phylogenetic tree shown here is part of a more comprehensive phylogenomic analysis shown in Fig. S1. *Number corresponds to JGI project ID.

In addition, a phenotypic comparison between '*Thiobacillus prosperus*' and the genera *Thioalkalivibrio* and *Acidiferrobacter* belonging to the family *Ectothiorhodospiraceae* is consistent with its placement in this group (Table 1).

Although phylogenomic evidence places '*Thiobacillus prosperus*' in a branch most closely related to members of the genus *Ectothiorhodospira* within the family *Ectothiorhodospiraceae*, in particular next to *Ectothiorhodospira haloalkaliphila*, we suggest that it should be designated a representative of a separate genus. This proposal is based on two types of evidence: 16S rRNA gene sequence comparison and ANI analysis. The full-length 16S rRNA gene sequence of '*Thiobacillus prosperus*' and those of *E. haloalkaliphila* (NCBI accession NR_044900) and *Ectothiorhodospira* sp. PHS-1 (EU590916) are only 91 and 93 % identical, respectively. In addition, the 16S rRNA gene sequence of '*Thiobacillus prosperus*' is only 90 % identical to that of *Acidiferrobacter thiooxydans* DSM 2392^T (NCBI accession AF387301), the first acidophilic member of *Ectothiorhodospiraceae* family described (Hallberg *et al.*, 2011). These percentage identities are not high enough (<95 %) to support an intragenus relationship (Kim *et al.*, 2014), thus '*Thiobacillus prosperus*' is neither a member of the genus *Ectothiorhodospira* nor the genus *Acidiferrobacter*.

In order to further investigate the relationship of '*Thiobacillus prosperus*' to the genus *Ectothiorhodospira*, an ANI analysis was undertaken. ANI reflects the similarity of 1 kb DNA sequence fragments. Use of the ANI calculator described by Goris and coworkers (2007) and implemented at *http://enve-omics.ce.gatech.edu/ani/*, yielded ANI values of 76–81 % between the '*Thiobacillus prosperus*' genome sequence and several other genomes from different members of family *Ectothiorhodospiraceae* including *E. haloalkaliphila* and *Ectothiorhodospira* sp. PHS-1 (Table S3). These values are not high enough (Konstantinidis & Tiedje, 2005; Richter & Rosselló-Móra, 2009) for '*Thiobacillus prosperus*' to be definitively considered a member of any of the currently described genera of the family *Ectothiorhodospiraceae*, supporting the proposal that '*Thiobacillus prosperus*' should be considered a representative of a novel genus and species. We propose the name *Acidihalobacter* gen. nov. for this new genus and suggest that the species name '*prosperus*' be retained.

Description of *Acidihalobacter* gen. nov.

Acidihalobacter [A.ci.di.ha.lo.bac′ter. N.L. n. *acidum* (from L. adj. *acidus* sour) an acid; Gr. n. *hals* sea, salt; N.L. masc. n. *bacter* rod; N.L. masc. n. *Acidihalobacter* an acid-loving, salt-loving rod].

Phylogenetic analysis on the basis of 16S rRNA gene and concatenated ribosomal protein sequences places the genus within the family *Ectothiorhodospiraceae* in the class *Gammaproteobacteria*. The type species is *Acidihalobacter prosperus*, and the genus description is based on that of the type species, currently the only species ascribed to this genus.

Table 1. Phenotype data comparison of *Acidihalobacter prosperus* gen. nov., sp. nov. (formerly '*Thiobacillus prosperus*') with other members of the family *Ectothiorhodospiraceae*

The compilation was retrieved from literature. All taxa are Gram-stain-negative. ND, Not detected; NR, not reported.

	*Acidihalobacter prosperus**	*Acidiferrbacter thiooxydans*†	*Ectothiorhodospira*‡	*Arhodomonas*‡	*Halorhodospira*‡	*Thiorhodospira*‡	*Thioalkalivibrio*‡
Flagellation	Polar flagellum	Non-motile	Polar tuft of flagella	Polar flagellum	Bipolar flagella	Polar tuft	Polar flagellum
Internal membranes	NR	NR	Lamellar stacks	ND	Lamellar stacks	Lamellar stacks	NR
Cell form	Straight rods	Slender straight rods	Vibrioid to spirilloid	Straight rods	Vibrioid to spirilloid	Vibrioid to spirilloid	Curved rods or spirilla
Cell width (μm)	3–4	2	0.7–1.5	0.8–1.0	0.5–1.2	3–4	0.8–3
Optimum NaCl requirement	0.4 M§	NR	1–7 %	15 %	15–25 %	0.5–1.0 %	5–10 %
Optimum pH for growth	2	2	7.6–8.5	6.5–7.5	8–9.2	9–9.5	10–10.2
Major quinone components	Q8	NR	MK7 and Q7 or Q8	NR	MK8, Q8 and MK4/5	NR	Q8
DNA G+C content (mol%)	64	63	61.4–68.4	67	50.5–69.7	56.0–57.4	61–65

*From Huber & Stetter (1989).
†From Hallberg *et al.* (2011).
‡From Imhoff (2005).
§Chloride concentration, evaluated for '*Acidihalobacter aeolicus*' V6 (see Davis-Belmar *et al.*, 2008).

Description of *Acidihalobacter prosperus* (Huber and Stetter 1989) sp. nov.

Acidihalobacter prosperus (pros′pe.rus. L. masc. adj. *prosperus* prosperous, referring to its ability to gain precious metals by mineral bioleaching).

This organism was initially assigned to the genus *Thiobacillus* based on classical phenotypic properties, as its 16S rRNA gene sequence was not available at the time. However, we propose that it is reclassified as *Acidihalobacter prosperus* on the basis of 16S rRNA gene and concatenated multiprotein phylogenetic analyses. An updated description of this organism is given. Cells are Gram-stain-negative rods, about 3 to 4 μm long and 0.3 μm in width, and are motile with one polar flagellum. Optimal growth occurs around 37 °C but the organism grows at up to 41 °C. Requires a minimum of 0.04 M Cl^- for growth but can grow with up to 3.5 % NaCl, and between pH 1.0 and 4.5. Strictly chemolithoautotrophic and aerobic. Grows on elemental sulfur and ferrous iron and on sulfidic ores like pyrite, sphalerite, chalcopyrite, arsenopyrite and galena, and on H_2S. Produces sulfuric acid from reduced sulfur compounds. Sensitive to ampicillin and vancomycin and possesses *meso*-diaminopimelic acid and ubiquinone Q-8. Lives in marine sediments in hydrothermal areas.

The type strain is DSM 5130^T (=JCM 30709^T). The G+C content of the DNA of the type strain is 64 mol%.

Acknowledgements

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