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

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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 5130T (=JCM 30709T) 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...
an opportunity to re-evaluate its taxonomic 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 ModelGenerator 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 invariant (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 (Ossandón 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).
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\(^a\) (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 & Rossello-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.

| Flagellation Internal membranes | Acidihalobacter prosperus* | Acidiferrobacter thiooxydans† | Ectothiorhodospira‡ | Arhodomonas§ | Halorhodospira∥ | Thiorhodospira¶ | Thioalkalivibrio¶
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<tbody>
<tr>
<td>Cell width (µm)</td>
<td>3–4</td>
<td>2</td>
<td>0.7–1.5</td>
<td>0.8–1.0</td>
<td>0.5–1.2</td>
<td>3–4</td>
<td>0.8–3</td>
</tr>
<tr>
<td>Optimum NaCl requirement</td>
<td>0.4 M§</td>
<td>NR</td>
<td>1–7 %</td>
<td>15 %</td>
<td>0.5–1.0 %</td>
<td>5–10 %</td>
<td></td>
</tr>
<tr>
<td>Optimum pH for growth</td>
<td>2</td>
<td>2</td>
<td>7.6–8.5</td>
<td>6.5–7.5</td>
<td>8–9.2</td>
<td>9–9.5</td>
<td>10–10.2</td>
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<tr>
<td>Major quinone components</td>
<td>Q8</td>
<td>NR</td>
<td>MK7 and Q7 or Q8</td>
<td>MK8, Q8 and</td>
<td></td>
<td></td>
<td>Q8</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>64</td>
<td>63</td>
<td>61.4–68.4</td>
<td>67</td>
<td>50.5–69.7</td>
<td>56.0–57.4</td>
<td>61–65</td>
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*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 H2S. 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 5130T (=JCM 30709T). The G+C content of the DNA of the type strain is 64 mol%.

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References


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