Order II. Acidithiobacillales ord. nov.

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A. di. o. b. al. la. lences. M. L. masc. n. Acidithiobacillus type genus of the order; -ales ending to denote order; M. L. fem. pl. n. Acidithiobacillales the Acidithiobacillus order.

The order Acidithiobacillales was circumscribed for this volume on the basis of phylogenetic analysis of 16S rDNA sequences; the order contains the families Acidithiobacillaceae and Thermithiobacillaceae.

Autotrophs utilizing reduced sulfur compounds; mesophilic or moderately thermophilic.

Type genus: Acidithiobacillus Kelly and Wood 2000, 513VP.

Family I. Acidithiobacillaceae fam. nov.

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A. di. o. b. al. la. ceaes. M. L. masc. n. Acidothiobacillus type genus of the family; -aceae ending to denote family; M. L. fem. pl. n. Acidithiobacillaceae the Acidithiobacillus family.

The family Acidithiobacillaceae was circumscribed for this volume on the basis of phylogenetic analysis of 16S rDNA sequences; the family contains the genus Acidithiobacillus (type genus).

Description is the same as for the genus Acidithiobacillus.

Type genus: Acidithiobacillus Kelly and Wood 2000, 513VP.

Genus I. Acidithiobacillus Kelly and Wood 2000, 513VP

DONOVAN P. KELLY AND ANN P. WOOD

A. di. o. b. c. l us. L. adj. acidus sour; Gr. n. thios sulfur; L. n. bacillus a small rod; M. L. masc. n. Thiobacillus small acid-loving sulfur rod.

Obligately acidophilic, aerobic, Gram-negative rods, 0.4 × 2.0 μm, motile by one or more flagella. Optimum growth below pH 4.0, using reduced sulfur compounds to support autotrophic growth. Some species oxidize ferrous iron or use natural and synthetic metal sulfides for energy generation; some species oxidize hydrogen. Optimum temperature 30–35°C for mesophilic species and 45°C for moderately thermophilic species. Contain ubiquinone Q-8. Member of the Gammaproteobacteria.

The mol% G + C of the DNA is: 52–64.

Type species: Acidithiobacillus thi oxidans (Waksman and Joffe 1922) Kelly and Wood 2000, 513 (Thiobacillus thi oxidans Waksman and Joffe 1922, 239.)

FURTHER DESCRIPTIVE INFORMATION

The characteristics of the four current recognized species of Acidithiobacillus are shown in Table BXII.γ.16. The properties, taxonomy, and differentiation of the genera of sulfur-oxidizing, chemolithoautotrophic, Gram-negative, rod-shaped bacteria are summarized in the chapter on the genus Thiobacillus which appears in Volume 2 Part C in this Manual. Some species of Acidithiobacillus grow chemolithoautotrophically on sulfide minerals, and A. ferrooxidans also uses the oxidation of ferrous iron [and probably uranium (IV) and copper (I) oxidation] as the source of metabolic energy. One strain of A. ferrooxidans (ATCC 21834) has been shown to have a circular chromosome (2.9 Mb) and one small plasmid (8.6 kb) (Irazabal et al., 1997). Other bacteria able to oxidize sulfide minerals or iron (II) that appear superficially similar to Acidithiobacillus, but that are taxonomically and biochemically very different, have been described. These include the Gram-negative genus Leptospirillum and the Gram-positive genera Acidimicrobium and Sulfolobus (Balashova et al., 1974; Golovacheva and Karavaiko, 1979; Harrison and Norris, 1985; Clark and Norris, 1996; Norris et al., 1996).

TAXONOMIC COMMENTS

This genus was created to accommodate the obligately acidophilic Thiobacillus species that were shown by 16S rRNA gene sequence analysis to fall into the Gammaproteobacteria (McDonald et al., 1997; Kelly and Wood, 2000).

The assignment of T. albertis to the new genus as Acidithiobacillus albertensis is very tentative. The 16S rRNA gene sequence, required for placement in a family in the Proteobacteria, is not yet available. Its relatively high mol% G + C value (61.5) and the possession of a tuft of flagella and a glycocalyx could indicate significant differences from the other three species included here. Unfortunately, the original isolate may have been lost from culture (B.M. Goebel, personal communication).

Since the first description of Thiobacillus ferrooxidans (Temple and Colmer, 1951), numerous organisms from mineral leaching environments have been described as strains of T. ferrooxidans, because they were extremely acidophilic, obligately chemolithoautotrophic with ferrous iron as an energy substrate, and could degrade pyrite and various other sulfide minerals. It became clear that selective culture for organisms exhibiting this growth phenotype actually resulted in the isolation of morphologically and genomically diverse strains (Harrison, 1982; Kelly and Harrison, 1989; Kondrat’eva and Karavaiko, 1997; Goebel et al., 2000).

Eight different DNA homology groups of isolates described as T. ferrooxidans were identified by Harrison (Harrison, 1982; Kelly and Harrison, 1989). One of these groups was subsequently identified as Leptospirillum, and DNA–DNA hybridization values between the group containing the type strain (ATCC 23270) and the other six groups ranged from 0–19% to 58–73%. Using PCR-based techniques to assess this genomic variability, similarity coefficients between various isolates ranged from nearly 0 to >98% (Novo et al., 1996), and while several culture collection isolates were very closely related to the type strain (ATCC 23270).
strain (ATCC 33020) was not taxonomically related to the others (Selenska-Pobell et al., 1998). It is, therefore, inevitable that some strains assigned to *Acidithiobacillus ferrooxidans* will in due course be reassigned to new species or genera.

*Thiobacillus concretivorus* (Parker, 1945) was once listed as a distinct species in *Bergey’s Manual* (Parker, 1957) and appeared in the Approved Lists of Bacterial Names (Skerman et al., 1980). It is, however, now recognized as a synonym of *T. thiooxidans* (now *Acidithiobacillus thiooxidans*) (Vishniac, 1974; Kelly and Harrison, 1989).

### List of species of the genus Acidithiobacillus

1. *Acidithiobacillus thiooxidans* (Waksman and Joffe 1922) Kelly and Wood 2000, 513VP ([*Thiobacillus thiooxidans* Waksman and Joffe 1922, 299.](#))

   *thio*ox.i.dans. *Ge. n. thio sulfur; M.L. v. oxido make acid, oxidize; M.L. part adj. *thiooxidans* oxidizing sulfur.*

   Short rods, single, paired or in short chains, 0.5 × 1.0–2.0 μm. Motile by means of a polar flagellum (Doetsch et al., 1967). Minute colonies (0.5–1.0 mm) grown on thiosulfate agar appear transparent or whitish yellow and clear on prolonged incubation; edges appear complete. This organism grows in liquid medium on S0, thiosulfate, or tetrathionate; tetrathionate agar appear transparent or whitish yellow and clear on prolonged incubation; edges appear complete. This organism cannot oxidize iron or pyrite but has been shown to grow on sulfur, thiosulfate, or tetrathionate; tetrathionate is accumulated transiently during growth on thiosulfate but not on sulfur. Intracellular sulfur granules have been observed in stationary phase cells. Obligate chemolithotroph and autotroph. Aerobic. Ammonium sulfate is used as nitrogen source. Optimum temperature: 28–30°C. Optimum pH: 3.5–4.0; growth range pH: 2.0–4.5. Isolated from extremely acidic soil adjacent to a sulfur stockpile. Probably occurs in other similar environments.

   *The mol% G + C of the DNA is: 61.5 (UV spectrum).*  
   **Type strain:** ATCC 35403.


   Rods, 0.45 × 1.2–1.5 μm. Motile by means of a tuft of polar flagella. Condensed glycocalyx present, extending outwards from outer membrane, apparently involved in cell adhesion to surfaces such as sulfur. This organism grows on sulfur, thiosulfate, or tetrathionate; tetrathionate is accumulated transiently during growth on thiosulfate but not on sulfur. Intracellular sulfur granules have been observed in stationary phase cells. Obligate chemolithotroph and autotroph. Aerobic. Ammonium sulfate is used as nitrogen source. Optimum temperature: 28–30°C. Optimum pH: 3.5–4.0; growth range pH: 2.0–4.5. Isolated from extremely acidic soil adjacent to a sulfur stockpile. Probably occurs in other similar environments.

   *The mol% G + C of the DNA is: 61.5 (UV spectrum).*  
   **Type strain:** ATCC 35403.


   Cells are short, motile, Gram-negative rods, 0.7–0.8 × 1.2–1.8 μm. Capable of chemolithoautotrophic growth with thiosulfate, tetrathionate, sulfide, sulfur, and molecular hydrogen; no growth with ferrous iron or sulfidic ores. Mixotrophic growth with tetrathionate and glucose or yeast extract. Colonies on tetrathionate agar are small, circular, convex, smooth, and transparent, with precipitated sulfur in their center. Growth occurs between 32 and 52°C and

### Table BXII.7 16. Basic characteristics of species of the genus *Acidithiobacillus*

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<tbody>
<tr>
<td>Mol% G + C</td>
<td>52</td>
<td>61–62</td>
<td>63–64</td>
<td>58–59</td>
</tr>
<tr>
<td>Cell size (μm)</td>
<td>0.5 × 1.0–2.0</td>
<td>0.45 × 1.2–1.3</td>
<td>0.7 × 1.2–1.8</td>
<td>0.5 × 1.0</td>
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<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Single polar flagellum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tuft of polar flagella</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Glycocalyx</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Carboxysomes</td>
<td>+</td>
<td>nd</td>
<td>nd</td>
<td>+^</td>
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<tr>
<td>Obligately chemolithoautotrophic</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Optimum pH</td>
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<td>2.0–2.5</td>
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</tr>
<tr>
<td>pH limits</td>
<td>0.5–5.5</td>
<td>2.0–4.5</td>
<td>1.0–3.5</td>
<td>1.3–4.5</td>
</tr>
<tr>
<td>Optimum temperature, °C</td>
<td>28–30</td>
<td>28–30</td>
<td>45</td>
<td>30–35</td>
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<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>–</td>
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*Grows mixotrophically with tetrathionate plus glucose or yeast extract.

^Under carbon dioxide limitation.