NOTE

***Leptospirillum* gen. nov. (ex Markosyan 1972), nom. rev., including *Leptospirillum ferrooxidans* sp. nov. (ex Markosyan 1972), nom. rev. and *Leptospirillum thermoferrooxidans* sp. nov. (Golovacheva et al. 1992)**

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The name *Leptospirillum ferrooxidans* is not in the Approved Lists of Bacterial Names (1980), nor has it been subsequently validly published. In accordance with the rules of the International Code of Nomenclature of Bacteria, the name *Leptospirillum* for the genus (gen. nov., nom. rev.) and *Leptospirillum ferrooxidans* for the species (sp. nov., nom. rev.) is revived here. The type species is *Leptospirillum ferrooxidans* strain L15^T (= DSM 2705^T). The second species in the genus is *Leptospirillum thermoferrooxidans* (Golovacheva et al. 1992) (type strain L-88^T; Institute of Microbiology, INMI, Moscow, Russia).

Keywords: *Leptospirillum ferrooxidans*, *Leptospirillum thermoferrooxidans*, revived name, iron(II) oxidation

Markosyan (1972) described a new acidophilic, ferrous-iron-oxidizing, vibrio-shaped bacterium, isolated from mine water of the Alaverda copper deposit in Armenia, for which he proposed the name *Leptospirillum ferrooxidans*. The genus and species name *L. ferrooxidans* was, however, not included in the Approved Lists of Bacterial Names (Skerman et al., 1980), nor has it been subsequently validly published. Markosyan sent the type strain, designated L15^T, to the Institute of Microbiology (INMI), Moscow, Russia, where it was used in studies on the autotrophic growth, morphogenesis and fine structure of cells (Balashova et al., 1974; Pivovarova et al., 1981). In 1983, G. A. Zavarzin, Moscow, received another culture of *L. ferrooxidans* strain L15^T from Markosyan, and sent subcultures to the DSMZ, where it was deposited as DSM 2705^T, and to A. P. Harrison, University of Missouri, Columbia, USA, who designated a derivative strain Z-2. Harrison used this in comparative studies with other isolates for DNA G+C content determinations and DNA–DNA hybridizations (Harrison, 1984; Harrison & Norris, 1985). Phylogenetic studies on strain L15^T have demonstrated its unique position, separate from *Thiobacillus ferrooxidans* as well as from any other division of the *Bacteria*, such as the *Proteobacteria*, Gram-positive bacteria and spirochaetes (Lane et al., 1992; Ehrlich et

al., 1995; De Wulf-Durand et al., 1997). Subsequently, additional strains were isolated which are morphologically and physiologically similar to *L. ferrooxidans* strain L15^T and were designated *Leptospirillum*-like bacteria. DNA–DNA hybridization studies and 16S rDNA analyses revealed, however, that the new isolates were not identical to strain L15^T, but may represent different species (Groudev et al., 1978; Harrison, 1984; Harrison & Norris, 1985; Helle & Onken, 1988; Merrettig et al., 1989; Sand et al., 1992; Hallmann et al., 1992; Goebel & Stackebrandt, 1994; Battaglia et al., 1994). The important role of leptospirilli in bacterial leaching of sulphidic minerals in acidic environments is well documented (Norris & Kelly, 1982; Merrettig et al., 1989; Sand et al., 1992; Hallmann et al., 1992; De Wulf-Durand et al., 1997; Battaglia et al., 1998).

Although the inability to utilize sulphur or thio-sulphate is a common property of *L. ferrooxidans* and *Leptospirillum*-like bacteria, pyrite is used for growth by *Leptospirillum*-like bacteria as shown, for example, by Sand et al. (1992) and Schippers et al. (1996) for strain R3 isolated from Ilba Mine in Romania, Helle & Onken (1988) for strain P₃A isolated in Peru, Merrettig et al. (1989) for a strain isolated from the Rammelsberg Mine in Germany and Battaglia et al. (1994) for strains

L6 and L8 isolated from a cobaltiferous pyrite enrichment culture. In contrast, *L. ferrooxidans* strain L15^T was originally described to be unable to oxidize iron of pyrite and chalcopyrite, but was capable of growth and oxidation of pyritic iron in mixed culture with sulphur-oxidizing thiobacilli only (Balashova *et al.*, 1974; Norris & Kelly, 1978). However, subsequently subcultures of strain L15^T were found to oxidize pyrite and chalcopyrite in pure culture after several weeks of adaptation (Norris & Kelly, 1982; W. Sand, personal communication).

A 2262 bp chromosomal DNA fragment from *L. ferrooxidans* strain Z-2, containing a chemoreceptor gene, has been cloned, sequenced and expressed in *Escherichia coli* (Delgado *et al.*, 1998).

The qualitative chemical composition of the LPS of *Leptospirillum*-like bacterium strain BU-1 was examined by Yokota *et al.* (1988), and 2-keto-3-deoxyoctanoate, rhamnose, heptoses and glucosamine, but not phosphorous, were found. The exopolymeric substances (EPS) excreted by strain R3 after growth on pyrite or iron sulphate, were shown to consist mainly of neutral sugars, some uronic acids and iron species (Gehrke *et al.*, 1995). As suggested by Sand *et al.* (1995), the EPS with ferric ions bound are a prerequisite for the attachment of leaching bacteria to the metal sulphide surface and the chemical attack.

Cell-free extracts of *L. ferrooxidans* strain L15^T contain ribulose biphosphate carboxylase activity, demonstrating the autotrophic nature of this organism (Balashova *et al.*, 1974). In ultrastructural studies, however, carboxysomes were not detected (Sand *et al.*, 1992).

Recently, a moderate thermophilic *Leptospirillum*-like strain (L-88^T), exhibiting a low level of DNA homology with *L. ferrooxidans* strain L15^T, was isolated from an acidic, iron-containing hydrothermal spring on Kunashir Island, Kuril Islands, Russia, and described as a new species, *Leptospirillum thermoferrooxidans* (Golovacheva *et al.*, 1992). Another moderate thermophilic strain, C-Lf30A, capable of growth up to 45 °C, has been isolated from a bioleaching reactor in Australia by Goebel & Stackebrandt (1994). A possible relationship to *L. thermoferrooxidans* has not been explored.

Here, the name *Leptospirillum* for the genus and the name *Leptospirillum ferrooxidans* for the species are revived, for the same organism for which this name was originally used by Markosyan (1972), in accordance with Rules 27, 28a and 33c of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992).

Description of *Leptospirillum* gen. nov. (ex Markosyan 1972), nom. rev.

Leptospirillum (lep'to.spi.ril'lum. Gr. adj. *leptos* thin, fine; Gr. n. *speira* a spiral; M.L. neut. n. *Leptospirillum* thin spiral).

Cells are vibrioid to spirilla-shaped, the latter consisting of 3–12 cells giving a *Spirillum*-like morphology; cocci and pseudococci can be formed. Vibrios measure 0.2–0.6 by 0.9–3.5 µm. Motile by means of a single polar flagellum. Gram-negative. Endospores not formed. Aerobic. Acidophilic; grows in a pH range of 1.3–4.0. Mesophilic or moderately thermophilic with an upper limit around 55 °C. Chemoautotrophic growth by oxidation of ferrous iron, but not of sulphur or thiosulphate. Pyrite and pyritic ore may or may not be utilized. The G+C content of the DNA is 50–57 mol %. The type species is *Leptospirillum ferrooxidans*. Currently, the genus *Leptospirillum* includes two species, *L. ferrooxidans* and *L. thermoferrooxidans*.

Description of *Leptospirillum ferrooxidans* sp. nov. (ex Markosyan 1972), nom. rev.

Leptospirillum ferrooxidans (fer.ro.ox'i.dans. L. n. *ferrum* iron; M.L. v. *oxido* oxidize; M.L. part. adj. *ferrooxidans* iron-oxidizing).

The description is based on the data for strain L15^T as reported by Markosyan (1972), Balashova *et al.* (1974), Harrison (1984) and Harrison & Norris (1985). Cells are small, curved rods measuring 0.3–0.6 by 1.0–3.3 µm. A peculiar growth cycle with the formation of pseudococci, vibrios and spirilla was observed. Non-spore-forming. Motile by means of a single polar flagellum. Gram-negative. Growth in acidic environment (pH 1.5–4.0) on mineral medium containing iron(II). Energy for growth gained from the oxidation of iron(II). Use of organic compounds for growth not observed. Sulphur compounds not used as an energy source. Aerobic, mesophilic, pH optimum at 2.5–3.0. The G+C content of the DNA is 51.7 mol % (HPLC). The type strain L15^T (= ATCC 29047^T = DSM 2705^T = VKM B-1339^T), from which strain Z-2 of Harrison (1984) is derived, was isolated from mines of the Alaverda copper deposit in Armenia.

Description of *Leptospirillum thermoferrooxidans* sp. nov. (Golovacheva *et al.*)

Leptospirillum thermoferrooxidans (ther.mo.fer.ro.ox'i.dans. Gr. n. *therma* warm; L. n. *ferrum* iron; M.L. v. *oxido* oxidize; *thermoferrooxidans* warm iron-oxidizing).

The description of *Leptospirillum thermoferrooxidans* is identical to that given by Golovacheva *et al.* (1992). The type strain is strain L-88^T, maintained in the collection of the Institute of Microbiology (INMI), RAS, Moscow, Russia.

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