Phylogeny and photosynthetic features of *Thiobacillus acidophilus* and related acidophilic bacteria: its transfer to the genus *Acidiphilium* as *Acidiphilium acidophilum* comb. nov.

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Phylogenetic analyses based on 16S rDNA sequences and genomic DNA–DNA relatedness showed that the sulphur-oxidizing facultative chemolithotroph *Thiobacillus acidophilus* was closely related to members of the genus *Acidiphilium*, which is a group of strictly aerobic, heterotrophic acidophiles now categorized into aerobic photosynthetic bacteria. Lipophilic pigment analyses revealed that zinc-chelated bacteriochlorophyll a and carotenoids occurred in appreciable amounts in *T. acidophilus* and all established species of the genus *Acidiphilium*. PCR experiments showed that *T. acidophilus* as well as *Acidiphilium* species contained *puf* genes, encoding the photosynthetic reaction centre proteins and the core light-harvesting complex of the purple bacteria. There were high similarities between *T. acidophilus* and *Acidiphilium* species in the primary structure of their reaction centre proteins deduced from the nucleotide sequence data. The phylogenetic tree of the reaction centre proteins was in agreement with the 16S rDNA sequence-based phylogenetic tree in the relationship between *T. acidophilus* and *Acidiphilium* species and between the *Acidiphilium* cluster and other purple photosynthetic bacteria. Based on these results, together with previous phylogenetic and phenotypic information, it is proposed to reclassify *T. acidophilus* (Guay and Silver) Harrison 1983 as *Acidiphilium acidophilum* comb. nov. The type strain is ATCC 27807T (= DSM 700T).

**Keywords:** *Thiobacillus acidophilus*, zinc-bacteriochlorophyll, photosynthetic reaction centre, phylogeny, *Acidiphilium acidophilum* comb. nov.

**INTRODUCTION**

The genus *Thiobacillus* is a group of obligately or facultatively chemolithotrophic aerobic proteobacteria that are capable of growing with reduced inorganic sulphur compounds as sole energy source, but, at this time, is quite heterogeneous with members exhibiting a wide range of physiological, chemotaxonomic and genetic characteristics (Katayama-Fujimura et al., 1982, 1983; Kelly & Harrison, 1989; Lane et al., 1985, 1992). This group encompasses a number of acidophilic species (Harrison, 1984; Pronk et al., 1990), including *Thiobacillus acidophilus*, in addition to neutrophilic thiobacilli. The name *T. acidophilus* was proposed by Guay & Silver (1975) for some strains of facultatively chemolithotrophic acidophilic thiobacilli, but did not appear on the Approved Lists of Bacterial Names (Skerman et al., 1980). Harrison (1983) revived the name *T. acidophilus* following his confirmation that this organism grew equally well with elemental sulphur or glucose as a sole energy source. Phylogenetic analyses based on SS rRNA sequences (Lane et al., 1985) and partial 16S rRNA sequences (Lane et al., 1992), however, indicated that *T. acidophilus* was far distant from any other *Thiobacillus* species and was closely related to species of the genus *Acidiphilium*, which includes aerobic

**Abbreviations:** BChl, bacteriochlorophyll; BPhe, bacteriopheophytin. The DDBJ accession numbers for the 16S rDNA sequences determined in this paper are D86508, D86509, D86511, D86513 and AB006712; for the *puf* gene the number is AB013379.
Acidiphilic chemo-organotrophic bacteria that are unable to use reduced sulphur compounds as energy source (Harrison, 1989; Kishimoto et al., 1995b). Despite the dissimilarity in sulphur metabolism between *T. acidophilus* and *Acidiphilium* species, the available phylogenetic information has strongly suggested that the former species should be positioned among members of the genus *Acidiphilium*.

Recent research has shown that *Acidiphilium* species can be categorized into a group of aerobic photosynthetic bacteria (Shimada, 1995) because of their production of bacteriochlorophyll (BChl) only under aerobic growth conditions and their photosynthetic activity (Kishimoto et al., 1995a; Wakao et al., 1993, 1994). Interestingly, it has been shown more recently that a representative member of *Acidiphilium*, *Acidiphilium rubrum*, contains a fully active photosynthetic system with zinc-chelated bacteriochlorophyll (Zn-BChl) a as the major pigment (Wakao et al., 1996). This was the first demonstration of the existence of natural photosynthesis using (bacterio-)chlorophylls containing a metal other than magnesium.

Further studies have shown that all established species of the genus *Acidiphilium* contain the *puf* operon (Nagashima et al., 1997b), which is an assemblage of genes encoding the proteins of the photosynthetic reaction centre (L, M and C subunits) and the core light-harvesting complex (α and β subunits) of the purple photosynthetic bacteria. The structure of the *puf* operon is well-conserved among species of these bacteria, and thus the PCR technique is applicable for detection of a conserved region of *puf* genes (Nagashima et al., 1997a, b).

These recent findings motivated us to re-evaluate the phylogenetic relationships between *T. acidophilus* and members of the genus *Acidiphilium* and to determine whether *T. acidophilus* has photosynthetic properties. A previous study undertaken to detect photopigments in *T. acidophilus* gave negative results (Kishimoto et al., 1995a), but our attempts to find Zn-BChl a and *puf* genes in *T. acidophilus*, as well as in all *Acidiphilium* species, have been successful. Phylogenetic analyses based on 16S rDNA sequences, genomic DNA–DNA relatedness and the L and M subunit proteins of the reaction centre demonstrated that there were close relationships between *T. acidophilus* and *Acidiphilium* species. These results led us to conclude that *T. acidophilus* should be transferred to the genus *Acidiphilium* as *Acidiphilium acidophilum* comb. nov.

**METHODS**

**Bacterial strains and cultivation.** *Thiobacillus acidophilus* ATCC 27897T (′ = type strain) was studied. The following strains were used as the reference organisms: *Acidiphilium angustum* ATCC 35903T, *Acidiphilium cryptum* ATCC 33463T, *Acidiphilium multivorans* AIU301T, *Acidiphilium rubrum* ATCC 35905T, *Acidiphilium organovorans* ATCC 43141T and *Acidiphilium* sp. strains St1-5 and St1-7. All strains with ATCC numbers were obtained from the American Type Culture Collection (Rockville, MD, USA). *A. multivorans* AIU301T was isolated from acidic mine drainage (Wakao et al., 1994). All other strains were isolated newly by us from acidic mine water. All test organisms were grown aerobically at 30 °C in GYS medium, a chemically defined medium (pH 7.5) which consisted of a mineral base RM2 (Hiraishi & Kitamura, 1984), 15 mM glucose as the sole carbon source and 0.03% (w/v) yeast extract as the growth factor. *T. acidophilus* was also grown chemolitho-trophically with elemental sulphur as the energy source as described by Harrison (1983). For chemical and genetic testing, cells were harvested by centrifugation from a culture at the late-exponential phase of growth, washed twice with sterile 50 mM phosphate buffer (pH 6.8) and pelletted. The cell pellets were used immediately for analysis or stored at −20 °C until they were analysed.

**Analysis of BChls.** Lipophilic pigments were extracted from fresh wet cells with acetone-methanol (7:2, v/v), evaporated in vacuum and analysed by reverse-phase HPLC with a Shimadzu Liquid Chromatograph LC-10A equipped with a Beckman Ultrasound ODS column (4.6 i.d. × 250 mm) in a column oven at 30 °C. Samples were eluted with methanol at a flow rate of 1 ml min⁻¹ and monitored with a photodiode array detector, Shimadzu SPD-10A, in a wavelength range of 350–500 nm. Post-run data analysis was performed with the Shimadzu CLASS-M10A program. For the identification and quantification of BChls, parameters for peak identification and calibration of detector response factors were set in the program on the basis of HPLC data on a known concentration of *A. rubrum* Zn-BChl a which had been determined spectrophotometrically (Wakao et al., 1996). BChl a and bacteriopheophytin (BPh) purified from a purple phototrophic bacterium, *Rhodobacter sphaeroides* DSM 158T, were also used as the standard pigments. Although zinc-chelated BChl should be called zinc-BPh more precisely, we used herein the term Zn-BChl for convenience.

**Analysis of carotenoids.** Pigments extracted as noted above were analysed by HPLC equipped with a μBondapak C18 column (5 mm i.d. × 100 mm) (Waters) and 480 nm. Carotenoid components were eluted with methanol at flow rate of 2 ml min⁻¹ and detected with a MCPD-3600 photodiode array detector (Otsuka Electronics) in a wavelength range of 250–600 nm (Takaichi & Shimada, 1992). For spectrophotometric measurement of carotenoids, the following extinction coefficients in methanol were used: 150 mM⁻¹ cm⁻¹ at 492 and 480 nm for spirilloxanthin and rhodovibrin, respectively. Major carotenoids were also purified by column chromatography on silica gel 60 (Merck). Molecular masses were determined by field-desorption mass spectrometry with a double-focusing gas chromatograph-mass spectrometer equipped with a field desorption apparatus (Hitachi). (Takaichi, 1993). Spirilloxanthin purified from a purple phototrophic bacterium, *Rhodospirillum rubrum* ATCC 11170T, was used as the standard.

**DNA–DNA hybridization.** Genomic DNA was extracted and purified by the method of Marmur (1961). DNA–DNA pairing studies were performed by the quantitative dot-blot hybridization method with biotin labelling and colorimetric detection as reported previously (Hiraishi et al., 1991).

**Analysis of 16S rDNA.** 16S rDNA gene fragments that corresponded to positions 8 to 1510 of *Escherichia coli* 16S rRNA (Brosius et al., 1978) were amplified directly from the cell lysate by PCR with *Taq* DNA polymerase (Takara International Journal of Systematic Bacteriology 48
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Shuoz) and a pair set of eubacterial universal primers 27f and 1492r (Lane, 1991). PCR products were treated with a chloroform/isoamyl alcohol mixture and purified by the PEG precipitation method (Kusukawa et al., 1991; Hiraishi et al., 1995). 16S rDNA was sequenced with a SequiTherm Long-Read Cycle sequencing kit (Epicentre Technologies) with fluorescent primers and analysed with a Pharmacia ALF DNA sequencer and a Perkin-Elmer ABI manufacturer’s instructions. The subcloned DNA sequences with a Pharmacia ALF DNA sequencer and a Perkin-Elmer ABI were excluded from the calculations. Multiple alignment of sequence was performed with the International Journal of Systematic Bacteriology 1987. Evolutionary cycle sequencing with primers previously described (Nagashima et al., 1997b). A 21 kb fragment that corresponded to a continuous nucleotide stretch between *pufB* and *pufM* was amplified with a pair set of primers, B140F (5’-TGCGCAGCTG-CGGTGC-3’) and MR (5’-CCATGCCTAGCCGCCAGA-3’). PCR experiments were also performed with other two pair sets of primers, B140F vs LS10R (5’-TGGAGCCAC-CAGCTC ACCAAGA-3’) and LS10F (5’-TGGTGGAAGGCG-GTGGCTCA-3’) vs MR, resulting in generation of a 12 kb fragment between *pufB* and *pufL* and of a 9 kb fragment between *pufL* and *pufM*. PCR products were treated with chloroform/isoamyl alcohol, purified by agarose gel electrophoresis and glass binding with a Takara EasyTrap version 2 kit and sequenced directly by fluorescent cycle sequencing with primers previously described (Nagashima et al., 1997b). The two fragments of 1-2 and 0-9 kb were also subcoloned by the TA cloning method (Marehuk et al., 1991) with a pT7Blue T-Vector kit (Novagen). Transformation of *E. coli* JM109 was carried out according to a standard manual of molecular cloning (Sambrook et al., 1989). Plasmid DNA was isolated and purified by using a Pharmacia FlexiPrep kit according to the manufacturer’s instructions. The subcoloned DNA sequences were determined by cycle sequencing with pUC/M13 and T7 promoter universal primers. All reactions were analysed with a Pharmacia ALF DNA sequencer and a Perkin-Elmer ABI 373A DNA sequencer.

**Phylogenetic analysis.** Sequence data were compiled with the GENETYX-MAC program package (Software Development). Multiple alignment of sequence was performed with the CLUSTAL W program (Thompson et al., 1991). Evolutionary distances were calculated by using Kimura’s two-parameter model (Kimura, 1980). Phylogenetic trees were reconstructed by the neighbour-joining method (Saitou & Nei, 1987), and the topology of trees was evaluated by bootstrapping with 1000 resamplings (Felsenstein, 1985). Alignment positions with gaps and unidentified bases were excluded from the calculations.

**RESULTS**

**16S rDNA sequence comparisons**

The phylogenetic relationships between *T. acidophilus* and *Acidiphilium* species were re-examined based on 16S rDNA sequences. Although the sequence data on *T. acidophilus* was already available from the DDBJ, EMBL and GenBank databases before this study, there were large numbers of undetermined positions. To ascertain the exact phylogenetic position of *T. acidophilus*, therefore, we determined nearly complete sequences of the 16S rDNA of *T. acidophilus* and related acidophilic organisms. Binary sequence comparisons indicated that the 16S rDNA of *T. acidophilus* was most closely related to the 16S rDNAs of *A. angustum* and *A. rubrum* at a similarity level of 98-3% (corrected distance = 0.0168). A neighbour-joining phylogenetic tree was constructed on the basis of the distance matrix data on the test strains and several reference bacteria (Fig. 1). The tree showed that *T. acidophilus* fell into a cluster of the genus *Acidiphilium* with *A. angustum* and *A. rubrum* as its closest relatives. The monophyly of the cluster of the genus *Acidiphilium* and of the subcluster of *T. acidophilus* with *A. angustum* and the related strains as the sister group was supported by nearly 100% levels of bootstrap confidence. The results of our phylogenetic studies supported the previous results on the phylogeny of *Acidiphilium* species and related acidophiles (Lane et al., 1985, 1992; Sievers et al., 1994; Kishimoto et al., 1995b).

**Genomic DNA relatedness**

Interrelationships between *T. acidophilus* and *Acidiphilium* species were also studied by genomic DNA–DNA hybridization assays (Table 1). The DNA of *T. acidophilus* had 18–24% binding levels to those of *A. angustum* and *A. rubrum* and 9–11% to those of other *Acidiphilium* strains tested. The low but significant levels of DNA–DNA relatedness between *T. acidophilus* and *A. angustum* or *A. rubrum* were in accordance with the results of the 16S rDNA-based phylogenetic analysis. A high level of similarity between *A. angustum* and *A. rubrum* in the 16S rDNA structure and genomic DNA relatedness, as reported here and elsewhere (Wakao et al., 1994; Kishimoto et al., 1995b), suggest synonymy of the two species names. In this study, ‘*Thiobacillus organoparous*’ (Markosyan, 1973), an acidophilic facultative chemolithotroph similar to *T. acidophilus*, was not used, since the former species is probably a synonym of the latter in view of the high level of DNA–DNA homology between the two (Katayama-Fujimura et al., 1983). The results of DNA–DNA hybridization assays indicate that *T. acidophilus* is phylogenetically related to, but distinct from, the previously known species of the genus *Acidiphilium*.

**Photosynthetic pigments**

Comparative HPLC assays of the lipid extracts of *T. acidophilus* and *A. rubrum* demonstrated that the former organism produced much smaller but appreciable amounts of photopigments than the latter (Fig. 2). By comparing HPLC elution times and absorption spectrum patterns (not shown) of the separated components, the main pigment of *T. acidophilus* was identified as Zn-BChl *a*. In all other test strains of *Acidiphilium*, Zn-BChl *a* as well as Mg-BChl *a* and BPhe were detected by HPLC. The contents of these photopigments varied significantly among the strains tested, whereas Zn-BChl *a* always predominated in all...
test organisms (Table 2). The molar ratio of Zn-BChl a:Mg-BChl a:BPhe in the test organisms was relatively constant; for example, the mean ratio of the pigments with a molar basis of BPhe was 13:2:1 in the high Zn-BChl producers *A. angustum* and *A. rubrum*. In the strains showing low contents of the pigments (i.e. *A. cryptum*, *A. multivorum* and *A. organovorum*), the relative contents of Mg-BChl a was lowered, and those of BPhe were increased compared to the high Zn-BChl producers. These may be due to pheophytinization of a small amount of the Mg-BChl a during the extraction of pigments or HPLC analysis. Possibly for this reason, Mg-BChl a was hardly detected in *T. acidophilus*.

HPLC experiments also revealed that *T. acidophilus* and all *Acidiphilium* species contained spirilloxanthin as the sole or predominant carotenoid component (Fig. 2 and Table 2). The major carotenoid component purified from these bacteria had absorption maxima at 317, 384, 464, 491 and 524 nm in methanol. This component showed an HPLC retention time corre-
Table 1. Genomic DNA relatedness between *Thiobacillus acidophilus* and members of the genus *Acidiphilium*

<table>
<thead>
<tr>
<th>Strain</th>
<th>DNA G+C content (mol%)</th>
<th>Hybridization (%) with labelled DNA from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ATCC 2789T</td>
</tr>
<tr>
<td><em>Thiobacillus acidophilus</em> ATCC 2789T</td>
<td>63.5*</td>
<td>100</td>
</tr>
<tr>
<td><em>Acidiphilium angustum</em> ATCC 35903T</td>
<td>63.4†</td>
<td>18</td>
</tr>
<tr>
<td><em>Acidiphilium cryptum</em> ATCC 33463T</td>
<td>67.3†</td>
<td>8</td>
</tr>
<tr>
<td><em>Acidiphilium multivorum</em> AIU301T</td>
<td>67.6†</td>
<td>10</td>
</tr>
<tr>
<td><em>Acidiphilium organovorum</em> ATCC 43141T</td>
<td>67.4†</td>
<td>10</td>
</tr>
<tr>
<td><em>Acidiphilium rubrum</em> ATCC 35905T</td>
<td>63.2†</td>
<td>22</td>
</tr>
</tbody>
</table>

* Cited from Katayama-Fujimura et al. (1983).
† Cited from Wakao et al. (1994).

maxima at 465, 489 and 521 nm. This component is known to be an intermediate in spirilloxanthin biosynthesis in the purple bacteria.

While the liquid culture of *T. acidophilus* looked colourless, the massive cell pellet harvested by centrifugation was pale brown, probably due to the production of the photopigments. Direct spectrophotometric measurement of the acetone/methanol extract (3 ml) from the cell pellet (wet wt 1 g) detected only a weak peak with an absorption maximum at 763 nm, which is characteristic of Zn-BChl a (Wakao et al., 1996).

**Structure of puf genes**

In the purple bacteria, the genes encoding the photosynthetic reaction centre proteins and the core light-harvesting complex form an operon called *puf*. This gene construction has been shown to be well-conserved not only among the species of the anaerobic photosynthetic bacteria but also in *Acidiphilium* species (Nagashima et al., 1997a, b). Using the PCR technique, DNA fragments between *pujB* and *pufM* that covered a continuous 2.1 kb stretch of the *puf* genes were successfully amplified from *T. acidophilus* cells and sequenced.

The primary sequence of the *puf* genes of *T. acidophilus* contained three ORFs corresponding to *pufA*, *pufL* and *pufM* (data not shown). The levels of amino acid sequence similarity between *T. acidophilus* and *Acidiphilium* species were 88–93% in the L and M subunits, whereas much lower levels of similarity were found between *T. acidophilus* and other photosynthetic bacteria (Fig. 3). It has been shown that the L and M subunits of *Acidiphilium* species have one characteristic replacement of an amino acid in the region around the special pair when the three-dimensional structures of the reaction centres of *Blastochloris viridis* (formerly *Rhodopseudomonas viridis*) (Deisenhofer et al., 1995) and *Rhodobacter sphaeroides* (Allen et al., 1987) are
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Table 2. Bacteriochlorophyll and carotenoid contents of Thiobacillus acidophilus and Acidiphilium species

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Pigment content (nmol g⁻¹ dry wt)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Zn-BChl a</td>
</tr>
<tr>
<td>Thiobacillus acidophilus ATCC 27897^T</td>
<td>2.3</td>
</tr>
<tr>
<td>Acidiphilium angustum ATCC 35903^T</td>
<td>180</td>
</tr>
<tr>
<td>Acidiphilium cryptum ATCC 33463^T</td>
<td>57</td>
</tr>
<tr>
<td>Acidiphilium multivorum ATCC 301^T</td>
<td>67</td>
</tr>
<tr>
<td>Acidiphilium organovorum ATCC 43141^T</td>
<td>67</td>
</tr>
<tr>
<td>Acidiphilium rubrum ATCC 35905^T</td>
<td>810</td>
</tr>
<tr>
<td>Acidiphilium sp. St1-5</td>
<td>170</td>
</tr>
<tr>
<td>Acidiphilium sp. St1-7</td>
<td>130</td>
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</tbody>
</table>

Fig. 3. Amino acid sequence similarity in the L and M subunits of the photosynthetic reaction centre between Thiobacillus acidophilus and other photosynthetic bacteria and alignment of a part of the L subunit amino acid residues around the special pair. Asterisks mark identical residues in all species compared. One characteristic replacement of amino acid specific to Acidiphilium is shown by an arrow.

Analysis of lithothropically growing cells

Since all established species of the genus Acidiphilium and the related new genus Acidocella (Kishimoto et al., 1995b) have been reported to be unable to grow with reduced sulphur compounds as energy sources (Harrison, 1989), it seemed curious that the sulphur-oxidizing bacterium *T. acidophilus* has close phylogenetic relationships to those acidophilic chemoorganotrophic bacteria. This situation led us to re-examine whether *T. acidophilus* truly can oxidize reduced sulphur compounds as energy sources for growth, and this gave positive results in agreement with the previous reports (Guay & Silver, 1975; Harrison, 1983; Norris et al., 1986; Pronk et al., 1990; Meuenberg et al., 1992).

We compared the 16S rDNA structure of the sulphur-grown cells with that of the heterotrophically grown cells of *T. acidophilus* by studying RFLP patterns with *HaeIII*, *HhaI*, *MspI* and *Rsd*. The resulting 16S rDNA-RFLP patterns of the two cultures were identical and matched completely with the computer-predicted RFLP profiles (not shown). The DNA-DNA binding level between chemooorganotrophically grown and chemolithotrophically grown cells of *T. acidophilus* was 100%. PCR amplification of the *puf* gene from the sulphur-grown cells was also successful. These findings excluded the possibility that *T. acidophilus* is a mixed culture of a chemooorganotrophic species and a chemolithotrophic one.

taken into account for comparison (Nagashima et al., 1997b). This is at Glu L168, at which histidine occurs in the purple bacteria in general. The occurrence of glutamic acid at this position was also the case in *T. acidophilus* (Fig. 3).

A phylogenetic tree of the reaction centre proteins was constructed on the basis of the present data (Fig. 4). The topography of the tree was similar to that of 16S rDNA sequence-based tree in the relationships be-
DISCUSSION

As reported here, the molecular genetic analysis based on 16S rDNA sequence and DNA–DNA relatedness clearly demonstrates that T. acidophilus falls into a cluster of the genus Acidiphilium with A. angustum and A. rubrum as its nearest phylogenetic neighbours. These molecular data are in agreement with the previous findings as to the phylogeny of T. acidophilus, Acidiphilium species and related acidophiles (Lane et al., 1985, 1992; Sievers et al., 1994; Kishimoto et al., 1995b). Although T. acidophilus is most closely related to A. rubrum, it is clear that this bacterium represents a distinct species within the genus Acidiphilium, in view of the DNA–DNA hybridization data. These results unequivocally warrant reclassification of T. acidophilus as a new distinct species of this genus from a phylogenetic point of view.

The genus Acidiphilium was first proposed to accommodate strictly aerobic, chemo-organotrophic, acidophilic bacteria that grow under strongly acidic conditions (pH 2–6) (Harrison, 1983). Later some members of this genus have been shown to produce BCHls during aerobic growth (Wakao et al., 1993, 1994; Kishimoto et al., 1995a). This finding, together with 16S rDNA-based phylogenetic information, has led to the emendation of the genus Acidiphilium concurrently with the transfer of the non-pigment-producing species to the new genus Acidocella.
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Table 3. Differential characteristics of Acidiphilium acidophilum comb. nov. and other Acidiphilium species

<table>
<thead>
<tr>
<th>Character</th>
<th>A. acidophilum</th>
<th>A. angustum/ A. rubrum</th>
<th>A. cryptum</th>
<th>A. multivorum</th>
<th>A. organovorum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of colonies</td>
<td>W, PB</td>
<td>Pink to red</td>
<td>W, PB</td>
<td>W, PB</td>
<td>W, PB</td>
</tr>
<tr>
<td>Growth factor required</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chemolithothrophic growth with:</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Sulphur</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thiouresulphate</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Carbon source utilization:</td>
<td></td>
<td></td>
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<tr>
<td>Fumarate</td>
<td>-</td>
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<tr>
<td>Succinate</td>
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<td>(+)</td>
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</tr>
<tr>
<td>Glutamate</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>62.9–63.5</td>
<td>63.2–63.4</td>
<td>67.3–68.3</td>
<td>66.2–68.1</td>
<td>67.4</td>
</tr>
</tbody>
</table>

(Kishimoto et al., 1995b). Recently, the main component of photopigments in A. rubrum has been found to be Zn-BChl a (Wakao et al., 1996). Moreover, molecular genetic analyses have shown that all previously known species of the genus Acidiphilium emend. contain pfh genes encoding proteins of the photosynthetic reaction centre and the core light-harvesting complex (Nagashima et al., 1997b). Thus, Acidiphilium species are now recognized as aerobic photosynthetic bacteria that are unique in containing Zn-BChl a.

In this context, we examined the photosynthetic properties of T. acidophilus compared with those of all established species of the genus Acidiphilium. T. acidophilus as well as all Acidiphilium species produced detectable amounts of Zn-BChl a and carotenoids as reported here. The structural genes encoding the photosynthetic reaction centre of T. acidophilus were also detected and found to be similar to those of Acidiphilium species in primary structures and amino acid replacement around the special pair in the L subunit. The phylogenetic tree deduced from amino acid sequences of the L and M subunits demonstrates close relationships of T. acidophilus to Acidiphilium species with A. angustum and A. rubrum as its closest relatives, being consistent with the topography of the tree based on 16S rDNA sequences noted above.

Thiobacillus acidophilus differs from all known Acidiphilium species in the chemolithotrophic metabolism with reduced sulphur compounds. Nevertheless, the ability of T. acidophilus to produce the photopigments with Zn-BChl a as the major component demonstrates its phenotypic similarity to members of the genus Acidiphilium, and this provides a firm basis for the taxonomic reassignment of T. acidophilus in that genus. Concurrent phenotypic studies have also shown that T. acidophilus and Acidiphilium species share a number of common characteristics useful for the circumscription of the genus but are distinguishable from each other in some phenotypes as described below. Thus, based on the phylogenetic and phenotypic information described here, we propose transfer of T. acidophilus (Guay and Silver) Harrison 1983 to the genus Acidiphilium as Acidiphilium acidophilum comb. nov. Diagnostic characteristics of this organism and other Acidiphilium species are shown in Table 3.

Following this proposal, the situation may call for emendation of the genus Acidiphilium, because this genus has previously been defined to accommodate only ‘non-sulphur’ chemo-organotrophic acidophiles (Kishimoto et al., 1995b). However, it is our view, at this time, that all members of the genus Acidiphilium need re-examining more thoroughly in terms of sulphur metabolism. A previous study suggested that A. cryptum was unable to use sulphur as the energy source for growth but that it had the capacity for sulphur oxidation (Harrison, 1983). More effective data on sulphur metabolism should be helpful for the emendation of the genus Acidiphilium in the future.

Description of Acidiphilium acidophilum [Thiobacillus acidophilus (Guay and Silver) Harrison 1983] comb. nov.

Acidiphilium acidophilum (a.ci.do’phi.lum. L. adj. acidus sour; M.L. neut. n. acidum acid; Gr. adj. philus loving; M.L. adj. acidophilum acid-loving).

The description of this species is based on information from Guay & Silver (1975), Harrison (1983), Katayama-Fujimura et al. (1982, 1983, 1984), Norris et al. (1986), Mason et al. (1987), Pronk et al. (1990) and this study. Cells are rod-shaped, 0.5–0.8 μm wide.
by 1.0–1.5 μm long, occurring singly, pairs and rarely chains. Non-spore-forming. Motile or non-motile. Gram-negative. Colonies on agar media are round, regular, convex, slightly translucent, and white to cream; cell pellets harvested by centrifugation shows pale brown. Strictly aerobic facultative chemolithotrophs and mixotrophs growing with elemental sulphur as an energy source and with oxygen as the terminal electron acceptor. Thiolsulphate, trithionate and tetrathionate also serve as electron donor. Neither sulphite, sulphide nor ferrous iron serves as electron donor. Do not denitriify. Polyhedral inclusion bodies (carboxysomes) are present in elemental sulphur-grown cells. Optimal growth occurs at 25–30°C (range, 10 35°C) and at pH 3.0–3.5 (range, pH 1.5–6.0). No growth factor is required. Usable carbon sources are: L-arabinose, D-xylene, D-ribose, D-glucose, D-galactose, D-fructose, sucrose, glycerol, Dmannitol, ethanol, glucose, L-malate, citrate, L-glutamate, L-histidine, L-proline, DL-aspartate. Methanol and n-propanol support weak growth. Not utilized are: L-sorbitose, L-rihamnose, D-mannose, D-maltose, lactose, cellobiose, trehalose, D-melibiose, raffinose, n-butanol, cyclohexanol, ascorbic acid, formaldehyde, sucrose, fructose, n-propyamine and pyruvate. Strong growth is found in strongly acidic environments including mine water. Type strain: ATCC 27807T. "Escherichia coli. Proc Natl Acad Sci USA 1397".

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