Characteristics of *Sulfobacillus acidophilus* sp. nov. and other moderately thermophilic mineral-sulphide-oxidizing bacteria

Paul R. Norris, Darren A. Clark, Jonathan P. Owen and Sara Waterhouse

**INTRODUCTION**

Acidophilic bacteria that are most active in oxidation of ferrous iron and mineral sulphides at about 45–50 °C (Brierley & Brierley, 1986; Norris, 1990) have been isolated from geothermal environments, mineral sulphide mines, coal and mineral spoil heaps, and commercial metal leaching dumps. Their growth on ferrous iron and mineral sulphides in medium containing yeast extract was described (Brierley & Le Roux, 1977; Golovacheva & Karavaiko, 1979) before it was found this supplement could be replaced by separate, defined sources of organic carbon (some amino acids) and reduced sulphur (Brierley *et al.*, 1978; Norris *et al.*, 1980). Autotrophic growth on ferrous iron was demonstrated when culture atmospheres were enriched with CO₂ (Marsh & Norris, 1983a) and confirmed by measurements of CO₂ incorporation (Wood & Kelly, 1983) and ribulose bisphosphate carboxylase/oxygenase activity (Wood & Kelly, 1985). Mineral sulphide dissolution during autotrophic growth of some strains (Marsh & Norris, 1983b) demonstrated a potential application of these bacteria in extraction of metals from mineral sulphide concentrates and indicated a capacity for significant biogeochemical activity in acidic environments.

One of the most studied strains has been named *Sulfobacillus thermosulfidoxidan* (Golovacheva & Karavaiko, 1979). Some variation in its morphology (rods and coryneforms) has been described (Golovacheva, 1979) but this has not been reported for otherwise apparently similar bacteria (Brierley, 1978; Ghauri & Johnson, 1991). Isolates from several locations have been examined and are described in this paper in order to establish unequivocally the characteristics of *Sulfobacillus*-like bacteria. The dissimilarity between the reported 16S rDNA sequences of the *S. thermosulfidoxidan* type strain (Tourova *et al.*, 1994) and of very similar bacteria is addressed.

**METHODS**

**Bacterial strains.** The strains examined are listed in Table 1. *Sulfobacillus thermosulfidoxidan* type strain VKM B-1269 was provided by G. Karavaiko (Institute of Microbiology, RAS, Moscow, Russia) in 1993. Strain TH1 was provided by N. W. Le Roux (then at DTI Warren Spring Laboratory, Stevenage, UK) in 1977. Strains YTF and THW were provided by D. B. Johnson (University of Wales, Bangor). All other strains were isolated in this laboratory. Strains LM1 and BC1 were referred to as strains LM and BC in the description of their isolation (Marsh & Norris, 1983a) and the number added subsequently as different types (e.g. strain LM2, BC13; Norris, 1990) were obtained from the original enrichment cultures or sample sites. Several strains were isolated from provided samples and details of sample site conditions were not available. Strain TH3 was isolated from a copper leaching dump sample that was provided from the New Mexico Institute of Technology, Socorro, by J. A. Brierley (current affiliation Newmont Metallurgical Services, Salt Lake City, UT, USA). It was designated strain TH3 because it appeared very similar (Norris & Barr, 1985) to another strain TH3 (Brierley, 1978) which was no longer available. Both of these TH3 strains were isolated from the same site. Strain ICP is another isolate of the TH3 type (Clark & Norris, 1996). Strain C-MT1 (Goebel & Stackebrandt, 1994) was not examined in this work, but is discussed. It was obtained from a mineral sulphide ore-leaching, continuous bioreactor and described as a
Table 1. Strains and sources of moderately thermophilic, ferrous-iron-oxidizing bacteria

The approximate date when laboratory cultures were established is indicated.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
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<tbody>
<tr>
<td><em>S. therma</em></td>
<td>Mineral sulphide ore deposit, Armenia (1977)</td>
</tr>
<tr>
<td><em>therma</em></td>
<td></td>
</tr>
<tr>
<td>THI</td>
<td>Thermal spring, Iceland (1972)</td>
</tr>
<tr>
<td>BC1</td>
<td>Coal spoil heap, Birch Coppice colliery, UK (1981)</td>
</tr>
<tr>
<td>ALV</td>
<td>Coal spoil heap, near Alvecestra, UK (1979)</td>
</tr>
<tr>
<td>NAL</td>
<td>Coal spoil heap, near Alvecestra, UK (1988)</td>
</tr>
<tr>
<td>LM1</td>
<td>Thermal spring, Iceland (1981)</td>
</tr>
<tr>
<td>TH3</td>
<td>Copper leach dump, New Mexico, USA (1984)</td>
</tr>
<tr>
<td>2B, 3B, 3C</td>
<td>Thermal spring, Iceland (1989)</td>
</tr>
<tr>
<td>N</td>
<td>Thermal spring, Yellowstone National Park (1989)</td>
</tr>
<tr>
<td>YTF</td>
<td>Thermal spring, Yellowstone National Park (1989)</td>
</tr>
<tr>
<td>THW</td>
<td>Coal spoil heap, Wales (1988)</td>
</tr>
<tr>
<td>ICP</td>
<td>Thermal spring, Iceland (1993)</td>
</tr>
</tbody>
</table>

moderately thermophilic, iron-oxidizing, Gram-positive bacteria.

**Culture conditions.** All cultures were grown in shaken flasks in a mineral salts medium containing, per litre, MgSO₄ ∙ 7H₂O, 0.5 g; (NH₄)₂SO₄, 0.4 g; K₂HPO₄, 0.2 g; and KCl, 0.1 g. When 50 mM ferrous iron was the substrate (FeSO₄ ∙ 7H₂O, 13.9 g l⁻¹), the medium was initially adjusted with H₂SO₄ to pH 1.7. The medium was supplemented with tetrathionate (K₂S₂O₇, 0.15 g l⁻¹) for autotrophic growth on ferrous iron because some moderately thermophilic, iron-oxidizing acidophiles require a source of reduced sulphur for growth (Brierley et al., 1978; Norris & Barr, 1985). The medium was adjusted initially to pH 2 when yeast extract (0.25 g l⁻¹) was the substrate, and to pH 3 when the substrate was elemental sulphur (5 g l⁻¹). Medium containing sulphur or yeast extract was supplemented with a trace of iron (FeSO₄ ∙ 7H₂O, 10 mg l⁻¹). Medium containing pyrite (40%, w/v, iron; particle size diameter < 75 μm) was adjusted to pH 2 before inoculation. Pyrite was added in two stages, 1% (w/v) at inoculation and 4% (w/v) when growth was established. Cultures growing autotrophically on ferrous iron, sulphur or pyrite were gassed with 5% (v/v) CO₂ in air.

**Growth assays.** As described previously (Marsh & Norris, 1983a; Wood & Kelly, 1983, 1984), growth on ferrous iron was followed by titration of residual substrate using ceric sulphate and phenanthroline/ferrous sulphate as indicator. Pyrite digestion was followed by measuring iron in supernatants of cultures grown autotrophically on ferrous iron. A Joel JEM-100S transmission electron microscope was used to view thin sections of cells which had been fixed in glutaraldehyde and stained with uranyl acetate using standard techniques.

**Electrophoresis.** Cell lysates were prepared by incubation with lysozyme (5 mg ml⁻¹) at 37 °C for 15 min and subjected to SDS-PAGE (Laemmli, 1970) using a 10% (w/v) polyacrylamide gel.

**DNA extraction and analyses.** Cells were grown with yeast extract as substrate, harvested by centrifugation and washed with distilled water. Cell pellets from cultures (10 l) were resuspended in 6 ml buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8). EDTA (0.25 M, pH 8, 3.75 ml) and 50 mg lysozyme were added. After incubation at 37 °C for 15 min, 125 μl proteinase K (20 mg ml⁻¹) and 3.75 ml SDS (10% w/v) were added and incubation continued until the suspension cleared. DNA was isolated and purified following a modification of the Marmur protocol (Johnson, 1991) and a CsCl centrifugation step before dialysis.

DNA from moderate thermophiles and DNA (Sigma) from *Clostridium perfringens* (26.5 mol% G + C), *Escherichia coli* (52 mol% G + C) and *Micrococcus luteus* (72 mol% G + C) was dialysed three times against diluted standard saline citrate (i.e. 15 mM NaCl, 1.5 mM trisodium citrate, pH 7). Melting curve mid-points (Tm) were determined using a Hewlett Packard automated DNA melt testing system and 8452A spectrophotometer. Unknown base compositions were calculated from the formula mol% G + C = mol% G + C of X = 2.08(Tm - Tm of X), where X was the DNA of known base composition (Owen & Hill, 1979). Means of duplicate Tm determinations were used in the calculations and the mol% G + C of each moderate thermophile was taken as the mean of the three values calculated with reference to values obtained for the three DNA standards.

DNA-DNA hybridization was carried out using a filter hybridization technique as described by Sharp & Williams (1988). The results are the means of duplicate hybridizations except for strain TH3, with which a single experiment was performed. Hybridization is expressed as percentage of homologous hybridization counts.

**Analysis of 16S rDNA sequences.** PCR products comprising 16S rDNA of strain BC1 and *S. therma* were generated using 27f and 1492r primers (Lane, 1991). Cloning was done using the TA Cloning Kit (Invitrogen). Sequencing was done using primer 357f (Lane, 1991), the downstream vector primer M13r (5'-CAGGAAACAGCTATGAC-3') and an upstream vector primer (5'-GGCCCTCTAGATGCAT-3'). An Applied Biosystems model 373A was used for automatic sequencing. Percentage similarities were calculated for aligned sequences between bases 28-260, 371-617 and 1200-1460 (E. coli sequence numbering), a total of 741 bases. Included in
Iron-oxidizing moderately thermophilic acidophiles alignments were sequences previously determined by Lane et al. (1992) for strain BC1 (GenBank accession numbers M79380, M79381 and M79382) and strain ALV (M79375, M79376 and M80290), by Tourova et al. (1994) for S. thermosulfidooxidans (Z21979), and by Goebel & Stackebrandt (1994) for strain C-MT1 (X75270).

RESULTS

Whole-cell protein electrophoresis profiles

Comparative electrophoresis of whole-cell proteins of several isolates of ferrous iron-oxidizing moderate thermophiles revealed three groups of strains (Fig. 1). Further comparisons have shown that the protein profile of strain BC1 matched those of Sulfobacillus thermosulfidooxidans and strains TH1, LM1 and 3C (data not shown). Profiles of most of the other isolates (strains ALV, NAL, 2B, 3B, THW, YTF and N) did not match that of strain BC1 (Fig. 1) and were also different therefore from other representatives of the group containing S. thermosulfidooxidans. Within the strain ALV group, protein profiles were similar. There were a few, reproducible differences, particularly in the region showing polypeptides of about 25 kDa apparent molecular mass, where major bands were not aligned in the protein profiles of strains ALV and NAL, and were absent from profiles of strains THW, 2B and 3B. The SDS-PAGE protein profile of strain TH3 was very different from those of strain BC1 and strain ALV group bacteria (Fig. 1).

DNA:DNA hybridization

Three groups of moderate thermophiles were evident among the strains examined (Table 2), confirming the divisions seen with the protein profiles. Only one species in each group was indicated. There was generally well over 70% DNA:DNA hybridization among isolates of the strain ALV group. Further experiments with DNA from strains NAL, 2B, THW and 3B (not performed in duplicate; data not shown) showed high levels of DNA:DNA hybridization among these strains, so the latter two can also be included in the single species of the strain ALV group. There was over 80% hybridization between DNA from strain BC1 and S. thermosulfidooxidans.

DNA G+C content

The range of G+C mol% values for S. thermosulfidooxidans (Table 3) could reflect the variety of subspecies as well as different procedures in laboratories. The value obtained for the type strain in this study, and all values given for the similar strains BC1 and TH1 (Table 3), indicated a G+C content of between 48 and 50 mol%.

Table 2. DNA:DNA relatedness among moderately thermophilic, ferrous-iron-oxidizing acidophiles

Results are expressed as percentages of the homologous hybridizations. ND, Not determined.

<table>
<thead>
<tr>
<th>Filter-bound DNA from:</th>
<th>^H-labelled DNA from:</th>
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<tbody>
<tr>
<td></td>
<td>S. th.*</td>
</tr>
<tr>
<td>S. th.*</td>
<td>100</td>
</tr>
<tr>
<td>BC1</td>
<td>90</td>
</tr>
<tr>
<td>ALV</td>
<td>ND</td>
</tr>
<tr>
<td>NAL</td>
<td>12</td>
</tr>
<tr>
<td>2B</td>
<td>ND</td>
</tr>
<tr>
<td>3B</td>
<td>ND</td>
</tr>
<tr>
<td>N</td>
<td>ND</td>
</tr>
<tr>
<td>TH3</td>
<td>3</td>
</tr>
</tbody>
</table>

* S. thermosulfidooxidans.
strains tended to increase in size from about 0.6 x 2-3.5 μm during autotrophic growth on ferrous iron to 0.8-1.8 x 3.6-5 μm during growth on ferrous iron plus yeast extract and heterotrophic growth with yeast extract as the sole substrate (Fig. 2). The length of individual cells observed by light microscopy was difficult to measure, with most of the longer forms, including the filamentous forms of strain ALV, comprising dividing cells which had not separated (electron microscopy observations, not shown). With yeast extract as sole substrate, some S. thermosulfidooxidans strains became shorter and swollen, particularly strain BC1 (Fig. 2b) and, as reported previously, strain TH1 (Norris et al., 1980). Although flagella were not observed with the cells prepared for microscopy, limited motility of most Sulfobacillus-like strains was evident, but only when they were growing autotrophically on ferrous iron.

As described previously (Brierley, 1978), strain TH3 was morphologically unique among the isolates. The cells were characteristically narrower (0.4 μm wide) than Sulfobacillus strains and often in filaments (Fig. 2f). However, strain TH3 was occasionally observed to grow as pairs of relatively short cells, particularly during exponential growth on yeast extract in well-agitated cultures, when the cells were also motile.

### Endospore formation

Endospores were observed in S. thermosulfidooxidans and in the strain ALV group bacteria. They were more commonly observed in cells under the relatively poor nutritional conditions of autotrophic growth on ferrous iron (Fig. 2), with only strain N also showing endospores during heterotrophic growth (Fig. 2e). Whether sporulation is more prevalent towards the stationary phase of growth in the presence of yeast extract has not been examined. Spores appeared mostly spherical and terminal in the strain ALV group bacteria and possibly slightly more oval with less swelling of the cell in the S. thermosulfidooxidans strains. Sections of sporulating cells of each type revealed a typical Bacillus forespore development and mature spore structure, with clearly visible cortex and spore coat layers (Fig. 3).

### Growth and ferrous iron oxidation

The three groups of moderate thermophiles had different capacities for autotrophic growth on ferrous iron, assuming that iron oxidation was directly related to growth. Ferrous iron oxidation by strain TH1 growing on ferrous iron and yeast extract has previously been correlated with growth (cell carbon and protein) (Marsh & Norris, 1983a). Iron oxidation by autotrophically growing strains BC1 and ALV has been correlated with CO₂ fixation (Wood & Kelly, 1983). S. thermosulfidooxidans isolates (e.g. the type strain and strain LM1; Fig. 4) were able to maintain an initially high rate of oxidation of 50 mM ferrous iron. A gradual decline in the rate of oxidation was more evident with strains ALV and NAL and there was virtually no exponential phase of iron oxidation during

### Morphology

Strains of the three groups of moderate thermophiles defined by whole-cell protein electrophoresis, DNA G+C content and DNA:DNA hybridization could be similarly grouped on the basis of their size and their morphological variation in response to growth conditions. Two strain ALV was in part an exception to the pattern, growing as chains of sometimes distorted cells when oxidizing ferrous iron (Fig. 2c), but reverting to regular rods during autotrophic growth on sulphur (not shown) and during heterotrophic growth on yeast extract. Other isolates of the strain ALV group varied little in form (0.5-0.8 x 3-5 μm). In contrast, S. thermosulfidooxidans

### Table 3. Chromosomal DNA base composition of moderately thermophilic, ferrous-iron-oxidizing acidophiles

<table>
<thead>
<tr>
<th>Strain</th>
<th>mol % G+C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This study</td>
</tr>
<tr>
<td>S. th.*</td>
<td>48.4</td>
</tr>
<tr>
<td>BC1</td>
<td>48.6</td>
</tr>
<tr>
<td>TH1</td>
<td>ND</td>
</tr>
<tr>
<td>ALV</td>
<td>55.3</td>
</tr>
<tr>
<td>NAL</td>
<td>56.2</td>
</tr>
<tr>
<td>N</td>
<td>55.1</td>
</tr>
<tr>
<td>TH3</td>
<td>67.7</td>
</tr>
</tbody>
</table>

ND, Not determined.

* S. thermosulfidooxidans.
† a, Golovacheva & Karavaiko (1979); b, Karavaiko et al. (1988); c, subspecies thermostolerans, Kovalenko & Malakhova (1984); d, subspecies asporogenes, Vartanyan et al. (1988); e, Harrison (1986); f, Clark & Norris (1996); g, Brierley et al. (1978); h, Ghauri & Johnson (1991).
growth of strains TH3 and ICP. In the presence of yeast extract, strains of all three groups oxidized 50 mM ferrous iron rapidly and completely (data not shown), as shown previously with strains BC1, ALV and TH3 (Norris & Barr, 1985).

**Growth and sulphur oxidation**

Growth of all isolates in the presence of yeast extract and sulphur resulted in acidification of the medium, though this was relatively weak in cultures of strains TH3 and ICP. Transfer through many serial cultures in medium containing sulphur but no yeast extract resulted in consistent, autotrophic growth of strains ALV, NAL, THW, N and YTF, with, typically, pH 1.5 being reached, as seen previously with strain ALV (Norris et al., 1986). Only strain 2B of the strain ALV type did not readily switch to autotrophic growth on sulphur. In contrast, growth of *S. thermostilpidooxidans*, strain BC1 and strain TH3 became progressively weaker through serial culture on sulphur in the absence of yeast extract, and autotrophic cultures could not be maintained.

**Growth on pyrite**

Growth of some bacteria on mineral sulphides is inhibited by agitation with high mineral concentrations unless cultures are allowed to become established under less severe conditions (unpublished results). Strains BC1 and N, and the pyrite enrichment culture from which strain N was isolated, were therefore grown with only 1% (w/v) pyrite initially (Fig. 5). The concentration of pyrite was then increased so that concentrations of potentially growth-inhibiting end products of pyrite oxidation, H$_2$SO$_4$ and ferric iron, were reached. Extensive pyrite dissolution occurred during autotrophic growth of all *Sulfobacillus*-like isolates tested (*S. thermostilpidooxidans*, strains BC1, LM1, N, NAL, THW, YTF) except strain ALV. After addition of 4% (w/v) pyrite to established cultures (Fig. 5), iron solubilization was more rapid than by strain BC1 (60 mg l$^{-1}$ h$^{-1}$) than by strain N (41 mg l$^{-1}$ h$^{-1}$).

**Heterotrophic growth**

Bacteria of the strain ALV group generally tended to grow more readily than *S. thermostilpidooxidans* strains when switched from autotrophic growth and they were also easier to maintain subsequently with yeast extract as the sole substrate. The mean doubling times (estimated from culture optical density increase) were between 6 and 8 h for the strain ALV group (strains ALV, NAL, 2B and N) and between 8 and 12 h for the *S. thermostilpidooxidans* strains (i.e. the type strain, strains BC1, 3C and LM1). The yields of strains of both groups (estimated from culture optical density) were approximately proportional to the yeast extract concentration between 0.1 g l$^{-1}$ and 0.5 g l$^{-1}$. However, the maximum yield of *S. thermostilpidooxidans* strains growing on yeast extract (0.25 g l$^{-1}$) was on average one-third less (OD$_{440}$ 0.15–0.2) than that of strain ALV group bacteria (OD$_{440}$ 0.3). The optimum pH for growth of most strains on yeast extract was 2–2.2, but the precise optima were not determined. The growth rate and yield of strain TH1 were considerably reduced at pH 1.5 and pH 3.0 in comparison to growth at pH 2 (data not shown). In contrast, acidophilic, moderately thermophilic, heterotrophic *Bacillus* or *Allicyclobacillus*-like isolates from the same environments as some of the iron-oxidizing bacteria generally grew with estimated doubling times of 1.5–2 h and with a higher optimum pH (P. R. Norris, unpublished results).

**DISCUSSION**

The *Sulfobacillus*-like bacteria examined were clearly divided into two groups on a range of criteria (protein profiles, G+C content, DNA:DNA hybridization, morphology, characteristics of autotrophic growth on ferrous iron and sulphur, and heterotrophic growth yield). Taking into account all of these features, the extreme similarity of isolates in the first group, with 48–50 mol% G+C, indicated that they belonged to a single species. It is proposed that this should be *Sulfobacillus thermostilpidooxidans*, notwithstanding the incompatible rDNA sequences of the type strain as given in the database and as determined in this work. A phylogenetic tree derived from database sequences has placed *S. thermostilpidooxidans* in a cluster with the acidophilic heterotroph *Allicyclobacillus* rather than with strains BC1 and ALV (Tourouva et al., 1994), with which it appears to have much more in common. Subspecies of *S. thermostilpidooxidans* have been described as *thermo tolerant* (Kovalenko & Malakova, 1984) and *asporogenes* (Vartanyan et al., 1988). The relatively low mol% G+C contents of these strains (Table 3) places them with *S. thermostilpidooxidans* rather than the strain ALV group of bacteria. The 81% DNA:DNA hybridization between sub-species *asporogenes* and *S. thermostilpidooxidans* confirms this placement (Vartanyan et al., 1988).

It is proposed that the second group of *Sulfobacillus*-like bacteria, with 55–57 mol% G+C, represent a new species, *Sulfobacillus acidophilus*. The two species shared little DNA:DNA relatedness but strain BC1 (*S. thermostilpidooxidans*) and strain ALV (*S. acidophilus*) are more closely related phylogenetically to each other than to any other species (Lane et al., 1992) and they share a similar general physiology. Similar difference spectra indicated principally $b$-type and $aa_3$ cytochromes in strain TH1 (*S. thermostilpidooxidans*) and strain ALV (*S. acidophilus*) (Barr et al., 1990). The mixotrophic behaviour of strains ALV and BC1 was also generally similar, with simultaneous utilization of glucose and CO$_2$ during growth on ferrous iron (Wood & Kelly, 1983), although yeast extract depressed CO$_2$ fixation by strain BC1 more than that by strain ALV and, during growth in the absence of an enhanced CO$_2$ concentration, glucose stimulation of ferrous iron oxidation by strain ALV was stronger than with strain BC1. The difference in stimulation by glucose under air was also seen with strains NAL (*S. acidophilus*) and LM1 (*S. thermostilpidooxidans*), the latter showing much less improvement in growth (Clark & Norris, 1996). Glucose was utilized primarily by the oxidative pentose phosphate pathway in strain ALV (Wood & Kelly, 1984). Strain
ALV has received most study (Marsh & Norris, 1983b; Wood & Kelly, 1983, 1984; Norris et al., 1986; Harrison, 1986), but this comparison of several isolates has shown that its morphology during growth on iron and its poor growth on pyrite were not typical of the species. Strain NAL, isolated from the same site as strain ALV, is proposed as the type strain.

Several *S. acidophilus* strains catalysed extensive and rapid dissolution of pyrite, though with strain N at least, the rate was still slower than with an *S. thermosulfidooxidans* strain (Fig. 5). There was also a tendency for the rate to decline more rapidly as the acidity and the ferric iron concentration increased during the mineral dissolution (Fig. 5). A difference in susceptibility of the two *Sulfobacillus* species to ferric iron end-product inhibition of ferrous iron oxidation was also indicated by the more rapidly declining oxidation rate during growth of *S. acidophilus* (Fig. 4). Much greater inhibition of strain ALV, in comparison to strain BC1, occurred when ferric iron was added to growth medium (Norris et al., 1988). The least extensive ferrous iron oxidation was seen during growth of strains TH3 and ICP (Fig. 4), and this has also been correlated with an increased sensitivity to ferric iron.

Fig. 2. For legend see facing page.
Iron-oxidizing moderately thermophilic acidophiles

Fig. 2. Moderately thermophilic, acidophilic bacteria observed by light microscopy. Growth was on ferrous iron autotrophically (top row), on ferrous iron plus yeast extract (middle row), or heterotrophically on yeast extract (bottom row). All to same scale; bars 5 μm. Column (a) *S. thermosulfidooxidans*, (b) strain BC1, (c) strain ALV, (d) strain NAL, (e) strain N, (f) strain TH3.

in comparison with that of *S. thermosulfidooxidans* strains (D. A. Clark & P. R. Norris, unpublished).

Strain TH3 was clearly not related to the *Sulfobacillus* species. A new genus has been proposed, *Acidimicrobiium*, with strain TH3 and strain ICP as isolates of its single species, *A. ferrooxidans* (Clark & Norris, 1996). Moderately thermophilic, ferrous-iron-oxidizing bacteria that appear distinct from *Sulfobacillus* and *Acidimicrobiium* species have also been isolated (e.g. strain LM2: Norris, 1990; Ghauri & Johnson, 1991) but remain to be fully characterized.

**Description of Sulfobacillus acidophilus sp. nov.**

*Sulfobacillus acidophilus* (a.ci.do'phi.lus) sp. nov. ML n. *acidum* an acid; Gr. adj. *philus* loving; ML adj. *acidophilus* acid-loving.

Gram-positive rods (0.5–0.8 × 3.0–5.0 μm) with spherical endospores. Optimum growth is at 45–50 °C and approxi-
Fig. 3. Endospore formation in strain NAL (a, b) and strain BC1 (c, d). Bacteria were grown autotrophically on ferrous iron. Bars, 0.5 μm.

G + C. Source: various acidic environments rich in iron, sulphur or mineral sulphides. Type strain: strain NAL (German Collection of Micro-organisms, DSM 10332).

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References


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