Genomic and Physiological Comparisons Between Heterotrophic Thiobacilli and *Acidiphilium cryptum*, *Thiobacillus versutus* sp. nov., and *Thiobacillus acidophilus* nom. rev.

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Acidiphilium cryptum grows in yeast extract media with or without elemental sulfur. The growth rate and the cell yield are not changed by the presence of sulfur, but the pH of the medium drops slightly when sulfur is present, presumably because of gratuitous sulfur oxidation. No growth occurs with sulfur alone. *A. cryptum* is an obligate chemoorganotroph. *Thiobacillus acidophilus* grows equally well with either elemental sulfur or glucose as a sole energy source; this organism is a facultative autotroph. Since the name *T. acidophilus* does not appear on the Approved Lists of Bacterial Names, it is revived here, and strain ATCC 27807 is designated the type strain. *Thiobacillus perometabolis* is considered a subspecies of *Thiobacillus intermedius* on the basis of close phenotypic similarity and deoxyribonucleic acid homology. *Thiobacillus* sp. strain A2 is a distinctive mixotroph that shows little deoxyribonucleic acid homology with other species of *Thiobacillus*. This organism is named *Thiobacillus versutus*, and strain ATCC 25364 is designated the type strain.

Aerobic, mesophilic, nonsporulating, gram-negative, rod-shaped bacteria which utilize elemental sulfur or compounds containing oxidizable sulfur as sources of energy were placed by Vishniac (20) in the genus *Thiobacillus* Beijerinck. Some of these organisms are obligate chemolithotrophs, and some are acidophilic (i.e., they grow when they are inoculated into media at pH ≤ 3).

The terms proposed by Rittenberg (15) are used here to denote nutritional categories. Obligate chemolithotrophs acquire their energy from inorganic compounds, and the carbon requirement of these organisms can be met solely with CO₂. Mixotrophs can utilize inorganic and organic compounds concurrently. Chemolithotrophic heterotrophs acquire their energy from an inorganic compound but require an organic substance to fulfill their carbon requirement. Obligate chemoorganotrophs acquire their energy from organic compounds.

The non-acidophilic, obligate chemolithotrophs in the genus *Thiobacillus* include *Thiobacillus thioparus* and *Thiobacillus neapolitanus*. These species grow best near a neutral pH and may grow at pH values above 9; they require inorganic sulfur as a source of energy.

The non-acidophilic mixotrophs include *Thiobacillus novellus* (a soil organism named and originally described by Starkey [17, 18] and further described by Taylor and Hoare [19]) and *Thiobacillus* sp. strain A2, which was described

by Taylor and Hoare (19). These two organisms can grow autotrophically with thiosulfate as the energy source, heterotrophically with organic compounds, or mixotrophically by using inorganic and organic compounds simultaneously. (They have also been referred to as facultative autotrophs.) *Thiobacillus intermedius* also can grow autotrophically or heterotrophically, but it grows poorly unless both thiosulfate and an organic substance are present (12). *Thiobacillus perometabolis*, as originally described by London and Rittenberg (13), cannot grow autotrophically, but it does utilize thiosulfate as a source of energy if a suitable organic substance is present to meet its carbon requirement. Under these circumstances this organism grows as a chemolithotrophic heterotroph; it can also grow heterotrophically, with organic substances providing both energy and carbon.

The acidophilic, obligate chemolithotrophs include *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*. These organisms grow best between pH 2 and pH 5; little if any growth occurs at pH 6. *T. thiooxidans* and *T. ferrooxidans* are mesophiles (like the other species cited above), growing best at temperatures ranging from 25 to 35°C. *T. thiooxidans* utilizes inorganic sulfur as a source of energy, whereas *T. ferrooxidans* can utilize Fe²⁺ in addition to sulfur as a sole source of energy. If there is a strain of either of these two species that can be cultivated in conventional batch culture with glucose in lieu of sulfur or

iron, it remains to be described adequately. Strains of *T. ferrooxidans* that have been claimed to grow with glucose in lieu of Fe^{2+} have been shown to be contaminated with extraneous microorganisms (1, 3, 7).

The thermophilic iron-oxidizing bacteria that have been isolated from copper mine leaching dumps (2), which are acidophilic, chemolithotrophic heterotrophs, require an organic supplement in order to grow at 55°C. Ferrous iron serves as a source of energy, and the organic compound fulfills a particular carbon requirement (14).

The acidophilic, facultative autotroph *Thiobacillus acidophilus* was named and described by Guay and Silver (3). This species was discovered as a contaminant in an allegedly pure culture of *T. ferrooxidans* strain TM. Although *T. acidophilus* grows autotrophically with elemental sulfur as a sole source of energy and CO_2 as the source of carbon, it grows equally well with glucose as the source of energy in the absence of sulfur. This organism is not able to utilize Fe^{2+} as a source of energy.

Acidophilic, obligate chemoorganotrophs have been encountered in the same environments that harbor acidophilic thiobacilli. One species, *Acidiphilium cryptum* (5), has been isolated from allegedly pure cultures of *T. ferrooxidans* (6, 7). Unlike *T. acidophilus* of Guay and Silver, another contaminant in cultures of *T. ferrooxidans*, *A. cryptum* is not able to utilize inorganic sulfur (or Fe^{2+}) as a source of energy in laboratory media, either with or without organic supplements. However, in this paper I demonstrate that in yeast extract this species can oxidize sulfur gratuitously, with neither its growth rate nor its cell yield affected by sulfur oxidation. Thus, although it is not a thiobacillus, *A. cryptum* nevertheless may be involved in mineral leaching in acidic mineral environments. Numerous gram-negative, acidophilic chemoorganotrophs physiologically similar to *A. cryptum* were isolated from acidic mine water and were examined extensively by Wichlacz and Unz (22), but these bacteria have not been named.

In this paper I summarize and extend the previous descriptions of mixotrophic species of *Thiobacillus*. The deoxyribonucleic acid (DNA) analyses described here are the beginning of a study of the relationships among these and other bacteria. In addition, species status is accorded *Thiobacillus* sp. strain A2^T (type strain), and the name *Thiobacillus acidophilus*, which was not included on the Approved Lists of Bacterial Names (16), is revived.

MATERIALS AND METHODS

Bacterial strains and maintenance of bacteria. The strains used in this study are listed in Table 1. *T.*

acidophilus and *A. cryptum* strains were maintained on agar slants stored at 5°C for 1 month between transfers. The maintenance medium used was basal salts medium 1 supplemented with 1 g of glucose per liter, 0.1 g of Trypticase soy per liter, and 12 g of agar per liter. (Double-strength agar and sugar were autoclaved separately and were added to double-strength basal salts solution [pH 3] after the solutions had cooled to 45°C.) *T. ferrooxidans* strain m-1 was maintained in liquid ferrous sulfate medium (6) at 5°C for 3 weeks between transfers. The other thiobacilli were maintained on solidified Trypticase soy-glucose (TSG) medium slants at pH 6.9 or on solidified basal salts medium 1 (pH 6.9) supplemented with 0.5 g of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ per liter.

Media. Two mineral salts solutions were used to prepare the growth media. Basal salts solution 1 contained (per liter) 2 g of $(\text{NH}_4)_2\text{SO}_4$, 0.5 g of K_2HPO_4 , and 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Basal salts solution 2 contained (per liter) 0.4 g of NH_4Cl , 0.2 g of K_2HPO_4 , 0.2 g of KH_2PO_4 , 0.4 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.3 ml of a trace element solution (21). The pH of each solution was adjusted with 1 N H_2SO_4 and NaOH. The basal salts solutions were autoclaved at 121°C for 15 min, either as 10-ml volumes in test tubes (17-mm internal diameter by 150 mm) or as 100-ml volumes in 500-ml Erlenmeyer flasks. Various supplements were added to the basal salts solutions and were either autoclaved together with the salts solutions or sterilized separately (if required) at 10 times the desired final concentration and added to the sterile, cooled basal salts solutions. The following supplements were used, individually and in mixtures, as the experiments required: Trypticase soy broth (dehydrated; BBL Microbiology Systems, Cockeysville, Md.), 0.1 g/liter; yeast extract (dehydrated; BBL), 0.1 to 1 g/liter; $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, 5 g/liter; glucose, 1 g/liter; mannitol, 1 g/liter; and powdered sulfur (sublimed sulfur; type S-591 Fisher Scientific Co., Pittsburgh, Pa.), 10 g/liter. Sulfur was added to the basal salts solutions and sterilized at 105°C (to prevent melting) for 1 h on 2 successive days. TSG medium contained (per liter) 3 g of Trypticase soy broth (dehydrated; BBL), 1 g of glucose, and 2 g of K_2HPO_4 ; the pH was adjusted to 7. If needed, media were solidified with 12 g of agar per liter.

To obtain the 10^{12} cells required for DNA extraction, each culture was grown in 10 liters of appropriate medium in a 23-liter carboy with forced aeration at 28°C. Mannitol medium (Table 1) contained 10 g of mannitol (reagent grade) in 10 liters of basal salts medium 1. Glucose medium (Table 1) contained 10 g of glucose (reagent grade) in 10 liters of basal salts medium 2. Thiosulfate medium (Table 1) contained 100 g of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in 10 liters of basal salts medium 2. Media in carboys were autoclaved at 121°C for 1 h. Sulfur and ferrous sulfate media (Table 1) contained, respectively, 100 g of powdered sulfur and 400 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, each in 10 liters of basal salts, and were prepared as described previously (6).

Assays for growth. Cell counts were made microscopically by using a Petroff-Hausser bacterial counting chamber. A Coleman model 9 Nephro-colorimeter was used to measure growth photometrically and was calibrated for measuring the cell masses of thiobacilli (dry weight) with known weights of cells, a procedure described previously (8). Solidified medium in petri

TABLE 1. DNA homologies among and DNA base compositions of strains of *Thiobacillus* and *Acidiphilium*

Test strain ^a	Energy source ^b	G+C content of DNA (mol %) ^c	% Homology to reference DNA from strain: ^d					ATCC 25364 (TSG)	ATCC 33463 (G)
			7p (M) ^c	m-1 (Fe)	ATCC 23370 (Th)	ATCC 15466 (Th)	ATCC 8093 (TSG)		
<i>T. acidophilus</i> 7p	M	63–64	100	0	0	0	0	0	7
	S		105	0	0	0	0	0	
<i>T. acidophilus</i> DSM 700R	G		91						9
<i>T. thioparus</i> ATCC 8158	Th	63	8	0	0	0	12		0
<i>T. thioparus</i> ATCC 23646	Th	65	0	0	0	0	0	10	0
<i>T. ferrooxidans</i> m-1	Fe	65	0	100	0	10			0
<i>T. perometabolis</i> ATCC 23370	Th	66	0	0	100	78	0	11	0
	TSG	66	0	0	95	69	0	6	
<i>T. intermedius</i> ATCC 15466	Th	67	0	0	56	100	0	11	0
	TSG	67	0	0	51	105	12	7	
<i>T. novellus</i> ATCC 8093	Th	68			0	8	100	0	
	TSG	68	16	8	0	7	100	0	0
<i>Thiobacillus</i> sp. strain A2 ^T (= ATCC 25364 ^T)	TSG	68	0	0	0	0	0	100	0
<i>A. cryptum</i> Lhet2 (= ATCC 33463)	G	70	14		0		7	0	100
<i>A. cryptum</i> T21	G	69	26						88

^a *T. acidophilus* 7p is a single-colony isolate from strain 7 (= ATCC 27807) of Guay and Silver (3). Strain 7 was obtained from O. Tuovinen, and strain DSM 700 was obtained from H. Hippe of the Deutsche Sammlung von Mikroorganismen. This strain was sent to the German collection by R. Guay. *T. ferrooxidans* m-1 (4) and *A. cryptum* Lhet2 (= ATCC 33463) were isolated in my laboratory (5, 7). Strain T21 was kindly supplied by J. A. Antheunisse, who received it from Arkesteijn and de Bont (1). The other strains were acquired from the American Type Culture Collection.

^b The DNAs were extracted from cultures in media containing the following energy sources: mannitol (M), powdered sulfur (S), glucose (G), thiosulfate (Th), ferrous iron (Fe), and TSG. See text for the compositions of these media.

^c Except for *T. acidophilus*, the G+C contents were determined by the melting point method (see text). The value for *T. acidophilus* was taken from reference 3. The results of assays performed in other laboratories are as follows: *T. acidophilus* 7p, 63.5 mol%, determined by reverse-phase, high-pressure liquid chromatography of enzymatically hydrolyzed DNA supplied by me from a glucose-grown culture (Gehrke et al., personal communication); *T. thioparus*, 62 to 66 mol%, determined by buoyant density (9); *T. ferrooxidans* m-1, 65.3 mol%, determined by buoyant density with DNA supplied by me (M. Mandel, personal communication); *T. perometabolis*, 67.9 mol%, determined by buoyant density (Mandel, personal communication); *T. novellus*, 68 mol%, determined by buoyant density (19); *Thiobacillus* sp. strain A2^T, 65 to 68 mol%, determined by buoyant density (19); *A. cryptum* Lhet 2, 69.4 mol%, determined by buoyant density with DNA supplied by me (Mandel, personal communication).

^d The reference DNAs were radiolabeled with tritium (see text).

^e Energy source of the culture from which the DNA was extracted. M, Mannitol; Fe, ferrous iron; Th, thiosulfate; G, glucose.

plates was used to monitor the purity of cultures and also to determine the viable cell population.

Analyses of DNA. The guanine-plus-cytosine (G+C) contents of DNAs were determined by the melting point method described previously (6). The G+C values shown in Table 1 are arithmetic means from at least two assays. The G+C content was determined for DNA from a particular strain cultivated with different energy sources to aid in confirming the purity of the cultures.

DNA homologies were determined by the previously described S1 method (6), which is based on the procedure of Johnson et al. (11). The reannealing temperature in most instances was 70°C. To simplify comprehension of Table 1, homology values of 6% or less are recorded as zero homology. My interpretation of the results followed the suggestion of Johnson (10), namely, that DNA from a test strain (Table 1) having

≤20% homology to DNA from a reference strain (Table 1) indicates that the test strain belongs to a species that is not related to the reference strain. Test DNA having 20 to 50% homology to reference DNA indicates that the test strain belongs to a species different from, although closely related to, the species of the reference strain. Test DNA having 50 to 70% homology to reference DNA indicates that the test strain is related to the reference strain at the subspecies level. Test DNA having ≥70% homology to reference DNA indicates that the test strain may be considered a member of the same species as the reference strain. These homology ranges have been derived from experience with several bacterial groups through correlations with phenotype (10) and serve as approximations to establish relatedness at the species level.

For the bacteria listed in Table 1, experiments in

TABLE 2. Autotrophic growth of *T. intermedius* ATCC 15466 and *T. perometabolis* ATCC 23370 in thiosulfate-containing media^a

Organism	No. of cells/ml after incubation for: ^b									No. of divisions per h ^c
	0 Days	2 Days	3 Days	4 Days	5 Days	6 Days	7 Days	8 Days	10 Days	
<i>T. intermedius</i> ATCC 15466	1.0 × 10 ⁶	1.0 × 10 ⁷	1.0 × 10 ⁷	2.0 × 10 ⁷	2.0 × 10 ⁷	6.0 × 10 ⁷	1.0 × 10 ⁸	1.0 × 10 ⁸	1.6 × 10 ⁸	0.04
<i>T. perometabolis</i> ATCC 23370	1.3 × 10 ⁶	1.0 × 10 ⁷	2.0 × 10 ⁷	8.0 × 10 ⁷	1.5 × 10 ⁸	1.2 × 10 ⁸	1.5 × 10 ⁸			0.06

^a Cultures were incubated at 28°C in 100-ml portions of liquid medium in 500-ml Erlenmeyer flasks on a rotary shaker. The medium used was basal salts medium 2 (lacking the trace element solution) supplemented with 1.0% Na₂S₂O₃ · 5H₂O and prepared with water that had been double distilled in glass. These data are from the fourth of a series of four 100-ml shake flask cultures.

^b The inoculum was from a 1-week-old shake flask culture (the third serial culture) in the same medium, and the initial number of cells per milliliter was estimated from a microscopic count assay of the inoculum culture corrected for dilution (a 1-ml inoculum was used). All assays were performed by determining a microscopic count with a Petroff-Hausser bacterial counting chamber.

^c Calculated from the slope of the growth curve plotted with the data given above.

which replicate samples of the same DNA or samples of DNA from the same strain cultivated in different media were used resulted in homology values of $\geq 90\%$ compared with reference DNA from the same strain. Therefore, a homology value of 90% suggests, but does not prove, identity at the clonal level. The precision of the method in the high range of homology ($\geq 90\%$) is ~ 5 to 8% homology. Therefore, strains differing by only one or a few genes cannot be distinguished by means of DNA homology.

RESULTS AND DISCUSSION

In Table 1 the strains used in this study are listed in order of increasing G+C content. *T. thioparus* and *T. ferrooxidans*, which are not phenotypically related to the other species, were included because their G+C contents fall in the same range. As anticipated, these species showed negligible homology with mixotrophic thiobacilli and with the chemoorganotrophic species *A. cryptum*.

London and Rittenberg (13) noted that *T. perometabolis* and *T. intermedius* are similar in phenotype, but concluded that these two organisms "differ enough in specific metabolic characteristics to make it unlikely that the new isolate is a laboratory artifact, a chance mutant of *T. intermedius* . . .". The accuracy of this insight is supported by the homology results shown in Table 1. If the two organisms differ by one or a few mutations, their DNAs should show nearly 100% homology (within the experimental precision of the method used, which is ~ 5 to 8% homology). However, data in Table 1 do indicate a close relationship. Based on findings obtained with other bacteria (10), these organisms are related at least at the subspecific level.

As originally described (13), *T. perometabolis* did not grow autotrophically with thiosulfate. However, this organism does possess potential for autotrophic growth. By using a heavy inoculum, an autotrophic variant was acquired. This variant was cultivated through four successive 1-ml transfers in 100 ml of thiosulfate medium (Table 2) (at least 28 cell generations). Thiosulfate-adapted *T. perometabolis* ATCC 23370 grew at a somewhat faster rate than *T. intermedius* ATCC 15466 (Table 2), and these two organisms are phenotypically distinct. For example, thiosulfate-adapted *T. perometabolis* ATCC 23370 grows well when it is inoculated into TSG medium or yeast extract-glucose medium, whereas *T. intermedius* ATCC 15466 grows poorly in these media unless thiosulfate is added. Neither strain grows when it is inoculated into organic media at pH ≤ 3 (Table 3). However, once growth has begun at a higher pH in medium containing thiosulfate, catabolic activity can reduce the pH below 2.5 (12).

Taylor and Hoare (19) compared *T. novellus* and *Thiobacillus* sp. strain A2^T and concluded

TABLE 3. Phenotypic traits of thiobacilli and *A. cryptum*

Organism	Growth in yeast extract-glucose medium at the following pH values ^a										Growth with powdered sulfur as the sole source of energy ^b	Growth with thiosulfate as the sole source of energy ^b	Growth with other substrate ^c
	2.1	3.0	3.8	4.9	5.5	5.8	6.3	7.3	8.2	8.8			
<i>T. acidophilus</i> 7p	+ ^d	+	+	+	+	±	—	—	—	—	+		
<i>T. perometabolis</i> ATCC 23370	—	—	+	+	+	+	+	+	—	—	± ^e	± ^e	
<i>T. intermedius</i> ATCC 15466	—	—	—	+ ^f	+ ^f	+ ^f	+ ^f	+ ^f	+ ^f	—	+ ^g	+	
<i>T. novellus</i> ATCC 8093	—	—	—	—	+	+	+	+	+	+	— ^g	+	—
<i>Thiobacillus</i> sp. strain A2 ^T (= ATCC 25364 ^T)	—	—	—	—	+ ^h	+ ^h	+ ^h	+	+	+	— ⁱ	+	+
<i>A. cryptum</i> Lhet2 (= ATCC 33463)	+	+	+	+	+	+	—	—	—	—	—	—	

^a Organisms were grown in 100-ml portions of basal salts medium containing 0.05% (wt/vol) yeast extract and 0.1% (wt/vol) glucose in 500-ml Erlenmeyer flasks on a rotary shaker at 28°C. Flasks inoculated with *T. intermedius* ATCC 15466 were supplemented with 0.5% (wt/vol) Na₂S₂O₃ · 5H₂O to improve the growth of this organism.

^b Organisms were grown in 100-ml portions of basal salts medium containing 1% (wt/vol) powdered sulfur or 0.5% (wt/vol) Na₂S₂O₃ · 5H₂O in 500-ml Erlenmeyer flasks and incubated on a rotary shaker at 28°C.

^c Data from Taylor and Hoare (19). The substrates tested were sucrose, *n*-butanol, butyrate, L-leucine, L-isoleucine, L-glutamate, L-aspartate, malate, succinate, and cyclohexane carboxylate. These compounds were the only organic nutrients in the culture medium.

^d +, Good growth; —, no growth; ±, poor growth or variable results.

^e London and Rittenberg (13), reported that *T. perometabolis* was not able to grow autotrophically in thiosulfate. However, using a large initial inoculum, I adapted this species to grow at least as well as *T. intermedius* when thiosulfate was used as the sole energy source.

^f Once growth started in media containing thiosulfate, catabolism of the thiosulfate caused the pH to drop to approximately 2.5, but growth could not be initiated in this medium at a pH of about 4.

^g Data from London (12).

^h The cells grew as large spheres (spheroplasts), but appeared normal at pH ≥6.8.

ⁱ Data from Taylor and Hoare (19).

that it was possible that these two organisms belong to the same species. However, phenotypically these two organisms are as distinct as most currently recognized species of *Thiobacillus*. The homology data (Table 1) confirm distinctiveness at the genomic level.

Ideally, before an organism is reported as belonging to a new, undescribed species, several strains of the taxon from various sources should be isolated, described, and compared. However, no simple procedure that preferentially selects for mixotrophic bacteria has been devised, and the discovery of these bacteria has been fortuitous. For this reason new species of mixotrophs have been recognized and described on the basis of single, chance isolates. A species is no less valid or important merely because a convenient procedure for its selective isolation is unavailable. *Thiobacillus* sp. strain A2^T was discovered by chance 14 years ago (19), and no isolate with a similar phenotype has been described to date. In fact, the phenotypic characterization of this strain is an example of descriptive rigor (19). The metabolic diversity and robustness of *Thiobacillus* sp. strain A2^T suggest wide distribution.

Therefore, the name *Thiobacillus versutus* is proposed for this organism. Derivation of the specific epithet and a formal description of this species are given below.

T. acidophilus and *A. cryptum* are acidophilic. (Complete descriptions of these species have been given by Guay and Silver [3] and Harrison [5], respectively.) The former grows equally well with either elemental sulfur or glucose as an energy source (3, 7). *A. cryptum*, on the other hand, cannot utilize sulfur as an energy source, with or without an organic supplement (5). These two species are genomically distinct (Table 1).

In earlier studies with *A. cryptum*, basal salts medium 2 supplemented with 0.01% Trypticase soy and 0.1% glucose was used. Langworthy (personal communication) found that *A. cryptum* grows faster and attains a higher cell population in yeast extract than in Trypticase soy. Therefore, *A. cryptum* was cultivated in yeast extract medium for comparison. This experiment showed that the cell yield was proportional to the concentration of yeast extract throughout the range from 0.01 to 0.1%. Culture lag was at a

minimum and the growth rate was at a maximum (ca. 0.05 division per h) at yeast extract concentrations of 0.02 to 0.04%. Acid was formed when sulfur was present, presumably because of sulfur oxidation. This activity, however, was gratuitous since it did not increase the growth rate or improve the cell yield (Table 4). In contrast, *T. acidophilus* utilized sulfur as a sole source of energy, and the addition of sulfur to the yeast extract medium increased the cell yield above the yield attainable in the yeast extract medium alone (at a yield-limiting concentration of yeast extract) (Table 4). The growth of *T. acidophilus* with sulfur slowed down as the pH dropped to about 2. However, neutralizing the acid permitted growth to continue.

From *T. ferrooxidans* ATCC 13598, Arkesteijn and de Bont (1) isolated acidophilic contaminants which they assumed to be *T. acidophilus*. (*A. cryptum* had not been described at the time of their study.) One strain (strain T21) from their laboratory was examined (Table 1). This strain is a strain of *A. cryptum* and may be identical to strain 13598het, which has been described previously (7) and which was isolated from the same source. Thus, workers in two laboratories have independently demonstrated that *T. ferrooxidans* ATCC 13598 is contaminated with an extraneous chemoorganotroph.

T. acidophilus was described and named by Guay and Silver (3), but this name does not appear on the Approved Lists of Bacterial Names (16). Accordingly, this name is here revived.

In summary, *T. perometabolis* ATCC 23370 and *T. intermedius* ATCC 15466 are physiologically similar, differing only slightly. This similarity has been confirmed by DNA homology stud-

ies, which demonstrate that the former organism is related to the latter at the subspecific level. On the basis of phenotype and DNA homology data, *Thiobacillus* sp. strain A2^T of Taylor and Hoare (19) is clearly a distinct *Thiobacillus*. Accordingly, the name *Thiobacillus versutus* sp. nov. (ver. su'tus. L. adj. *versutus* versatile) is proposed for this organism. Strain A2 (= ATCC 25364) is designated the type strain. The following description of this species and of the type strain is taken largely from Taylor and Hoare (19).

T. versutus is an aerobic, mesophilic, gram-negative, nonsporulating, nonmotile, rod-shaped, non-acidophilic mixotroph. The G+C content of its DNA is approximately 68 mol%. It grows well in mineral salts medium with thiosulfate as the sole energy source, and it grows optimally between pH 7 and 9. This organism grows anaerobically in the presence of nitrate as an electron acceptor and produces copious N₂ with organic substrates, but not with thiosulfate in mineral salts medium. In yeast extract-glucose medium the growth rate is optimum between pH 7 and 9; growth is less rapid between pH 5.5 and 6.5, and the cells may become bloated; growth does not occur in this medium at pH <5. *T. versutus* can be differentiated from other non-acidophilic mixotrophic thiobacilli by its rapid rate of growth and cell yield in thiosulfate and organic media, by its denitrifying activity with organic substrates, and by its versatility in utilizing various organic compounds as sole sources of energy. The following compounds are utilized by *T. versutus* but not by *T. novellus* as sole energy sources: sucrose, butyrate, *n*-butyl alcohol, L-leucine, L-isoleucine, L-tryptophan, DL-serine, L-glutamate, L-asparagine, malate, succinate, citrate, benzoate, *p*-hydroxybenzoate,

TABLE 4. Growth of *T. acidophilus* DSM 700R and *A. cryptum* ATCC 33463 with and without elemental sulfur^a

Organism	Medium ^b	Initial no. of cells/ml ^c	Initial pH	Final no. of cells/ml ^d	Final pH ^e
<i>T. acidophilus</i> DSM 700R	Y	1 × 10 ⁶	4.1	8.0 ± 0.2 × 10 ⁷	5.2
	Y+S	1 × 10 ⁶	4.1	2.4 ± 0.2 × 10 ⁸	1.7
	S	1 × 10 ⁶	3.9	2.2 ± 0.2 × 10 ⁸	1.7
<i>A. cryptum</i> Lhet2 (= ATCC 33463)	Y	1 × 10 ⁶	4.1	2.0 ± 0.2 × 10 ⁸	5.7
	Y+S	1 × 10 ⁶	4.1	2.0 ± 0.2 × 10 ⁸	3.0
	S	1 × 10 ⁶	3.9	<5 × 10 ⁶	3.6

^a Cultures were incubated for 2 weeks at 28°C in 100-ml portions of liquid medium in 500-ml Erlenmeyer flasks on a rotary shaker.

^b The medium used was basal salts medium 1 supplemented with 0.02% yeast extract (Y) or 1% sublimed sulfur (S) or both.

^c The inocula (ca. 0.5 ml) were from shake flask cultures in basal salts medium 1 supplemented with 0.02% yeast extract and 0.1% glucose.

^d These cell yields, which were determined by microscopic counting, were attained within 1 week and remained unchanged during the second week of incubation (see text) (2 × 10⁸ cells is equivalent to 44 ± 4 µg [dry weight]).

^e The pH of uninoculated (control) flasks did not change during the 2-week test period (i.e., the final pH was the same as the initial pH).

m-hydroxybenzoate, *p*-aminobenzoate, and cyclohexane carboxylate.

T. acidophilus of Guay and Silver (3) is a distinctive facultative autotroph. Since this name also does not appear on the Approved Lists of Bacterial Names (16), it is revived here. A description of this organism, taken primarily from Guay and Silver (3), follows.

Thiobacillus acidophilus nom. rev. (a.ci. do'phi.us. L. adj. *acidus* sour; M. L. neut. n. *acidum* acid; Gr. adj. *philus* loving; M. L. adj. *acidophilus* acid loving). *T. acidophilus* is an aerobic, mesophilic, gram-negative, nonsporulating, motile or nonmotile, rod-shaped, acidophilic, facultative autotroph. The G+C content of its DNA is 63 to 64 mol%. It grows equally well in mineral salts medium with either elemental sulfur or glucose as the sole source of energy. It cannot use Fe²⁺ as a sole source of energy. Optimum growth in media containing these substances occurs between pH 2 and 4.5. Little if any growth occurs at pH ≥6. A variety of organic compounds can serve as sources of energy in a mineral salts medium; these include D-glucose, D-fructose, D-galactose, D-mannitol, D-xylose, D-ribose, L-arabinose, sucrose, citrate, malate, DL-aspartate, and DL-glutamate. The type strain is strain ATCC 27807. The description of the type strain is the same as that given above for the species.

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