Reidentification of *Thiobacillus perometabolis* ATCC 27793 and *Thiobacillus* sp. Strain A2 with Reference to a New Species, *Thiobacillus rapidicrescens* sp. nov.

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Thiobacillus perometabolis THI 024 (= ATCC 27793) is closely related to *Thiobacillus* sp. strain A2^T (type strain) (= THI 041^T = ATCC 25364^T), and these strains clearly differ from the known *Thiobacillus* species in their morphological and physiological characteristics, ubiquinone systems, deoxyribonucleic acid guanine-plus-cytosine contents, and cellular fatty acid compositions. We propose the name *Thiobacillus rapidicrescens* sp. nov. for these organisms. Strain THI 041 is the type strain of this species.

Thiobacillus sp. strain A2^T (type strain) has been investigated mainly with respect to its facultatively chemolithotrophic characteristics (2, 7, 12, 18); however, this strain has not been correctly identified since the isolation of the organism was reported by Taylor and Hoare in 1969 (14). In the course of our taxonomic studies on *Thiobacillus* species, we found that there is a close relationship between *Thiobacillus* sp. strain A2^T (= THI 041^T = ATCC 25364^T) and *Thiobacillus perometabolis* THI 022 (= ATCC 27793) (5).

In a previous paper (5), we classified *Thiobacillus* species into three groups according to the ubiquinone systems in their respiratory chains, their cellular fatty acid compositions, and the guanine-plus-cytosine (G+C) contents of their deoxyribonucleic acids. In this classification scheme *Thiobacillus* sp. strain A2^T was a member of group I, which included facultatively chemolithotrophic *Thiobacillus* species with ubiquinone 10; this group also contained *T. perometabolis* THI 022 and *Thiobacillus novellus* THI 031^T (group I-1). These organisms had octadecenoic acid plus cyclopropane acid of C₁₉ as their major non-hydroxylated cellular fatty acids, 3-hydroxydecanoic acid as a hydroxylated fatty acid, and G+C contents of 67.0 to 67.8 mol%. (Hexadecenoic acid and cyclopropane acid of C₁₇ are presented together in our study [5], as are octadecenoic acid and cyclopropane acid of C₁₉.) No hydroxylated fatty acid was detected in *T. novellus* THI 031^T. In contrast, *T. perometabolis* type strain THI 023 (= ATCC 23370) (8) was a member of group II, which included facultatively chemolithotrophic *Thiobacillus* species with ubiquinone 8. This organism had hexadecanoic acid, hexadecenoic acid

plus cyclopropane acid of C₁₇, and octadecenoic acid plus cyclopropane acid of C₁₉ as its major non-hydroxylated cellular fatty acids, 3-hydroxydecanoic acid and 3-hydroxydodecanoic acid as hydroxylated fatty acids, and a G+C content of 65.0 mol%.

We identified some physiological characteristics of *T. perometabolis* THI 022 which clearly differed from the characteristics given in the original description (10). Therefore, we obtained the culture deposited with the American Type Culture Collection, Rockville, Md., under accession number ATCC 27793 to confirm the results described above, and we designated this organism *T. perometabolis* THI 024 for this study.

In this paper we present a detailed comparison of cultures and propose a new species, *Thiobacillus rapidicrescens*, for *T. perometabolis* strain THI 024 and *Thiobacillus* sp. strain A2^T.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. The group I *Thiobacillus* strains were maintained on BY agar slants and transferred once a month. *T. perometabolis* THI 023^T was maintained on SM basal salts medium (pH 6.0) supplemented with 5% (wt/vol) sodium thiosulfate and 5% (wt/vol) yeast extract (Difco Laboratories, Detroit, Mich.) and was transferred once a week.

T. perometabolis THI 024 newly received from the American Type Culture Collection was similar to strain THI 022 in its morphological and physiological characteristics, its ubiquinone, its cellular fatty acid composition, and the G+C content of its deoxyribonucleic acid. Therefore, we present data for strain THI 024 below.

Bacterial names which are not on the Approved Lists of Bacterial Names (11) or have not been validly

TABLE 1. Bacterial strains studied

Group ^a	Designation	Received as: ^b	Source	Remarks
I	THI 022	<i>T. perometabolis</i> ATCC 27793		Received on 6 April 1978
	THI 024	<i>T. perometabolis</i> ATCC 27793		Received on 29 August 1980
	THI 041 ^T	<i>Thiobacillus</i> sp. strain A2 ^T (= ATCC 25364 ^T)		
	THI 031 ^T	<i>T. novellus</i> IAM 12100 ^T	ATCC 8093 ^T	
II	THI 023 ^T	<i>T. perometabolis</i> IAM 12132 ^T	ATCC 23370 ^T	

^a See reference 5.

^b ATCC, American Type Culture Collection, Rockville, Md.; IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan.

published since 1 January 1980 are enclosed in quotation marks.

Identification methods. The morphological characteristics of most strains were examined by using cells grown in BY medium; *T. perometabolis* THI 023^T was grown in SM basal salts-thiosulfate-yeast extract medium. BY medium contained 5 g of meat extract (Kyokuto, Tokyo, Japan), 5 g of peptone (Kyokuto), 5 g of yeast extract (Kyokuto), 2.5 g of NaCl, 0.1 g of K₂HPO₄, 0.05 g of MgSO₄ · 7H₂O, and 1,000 ml of tap water; the pH of this medium was 7.2 (3).

Motility was tested by using cultures grown in liquid BY medium or SM basal salts medium supplemented with thiosulfate with or without yeast extract at 30°C. Flagellation and cell size were confirmed by using cells cultured overnight on agar slants after negative staining with 1% (wt/vol) phosphotungstic acid and a model JEOL 200CX transmission electron microscope at 100 kV.

Physiological characteristics were examined in SM basal salts medium supplemented with adequate substrates by using methods described previously (3). SM basal salts medium contained 1.5 g of KH₂PO₄, 4.5 g of Na₂HPO₄, 0.1 g of MgSO₄ · 7H₂O, 0.3 g of NH₄Cl, 5 ml of a trace metal solution (17), and 1,000 ml of deionized water; the pH of this medium was adjusted to 7.5 (5). SM basal salts medium (pH 6.0) was used for the experiments with *T. perometabolis* THI 023^T. One loopful of cells was suspended in 10 ml of sterilized water, and this suspension was used as the inoculum (final concentration, 1%) in fresh liquid medium.

The ability to assimilate inorganic sulfur compounds was examined in liquid SM basal salts medium containing sodium thiosulfate (5 g/liter), elemental sulfur (10 g/liter), or potassium tetrathionate (3 g/liter). These substrates were autoclaved separately and added to the basal salts medium. Powdered sulfur was sterilized by intermittent steaming. Assimilation was determined on the basis of growth, changes in the pH of the media, decreases in the amounts of substrates, and resultant formation of sulfate during successive transfers in media of the same composition. Utilization of potassium thiocyanate (100 mg/liter) was examined in the medium described previously (3).

Assimilation of organic compounds was examined heterotrophically by using agar plates, as described previously (3). In addition, the assimilation test for *T. perometabolis* THI 023^T was carried out by using heterotrophic media and mixotrophic media which were supplemented with 5% (wt/vol) sodium thiosulfate. Assimilation ability was determined after 2 weeks

of incubation by comparing the sizes of the colonies on a test plate containing the substrate with the sizes of colonies on a control plate which contained only the basal salts medium.

Analytical methods. Thiosulfate and tetrathionate were analyzed by the method of Kelly et al. (6). Sulfate was determined gravimetrically after the addition of a 5% (wt/vol) BaCl₂ · 2H₂O solution in 0.1 N HCl. Potassium tetrathionate was prepared as described by Trudinger (15). The other chemicals used were available commercially.

RESULTS

Morphological and physiological characteristics. Table 2 shows the morphological and physiological characteristics of *Thiobacillus* sp. strain A2^T, *T. perometabolis* THI 024, *T. novellus* THI 031^T, and *T. perometabolis* THI 023^T.

Thiobacillus sp. strain A2^T and *T. perometabolis* THI 024 were motile by tufts of polar flagella, as shown in Fig. 1. *T. perometabolis* THI 023^T had a single polar flagellum (Fig. 1) (8). Motility was not observed in *T. novellus* THI 031^T (3, 13).

Thiobacillus sp. strain A2^T and *T. perometabolis* THI 024 could grow anaerobically in the presence of nitrate in SM basal salts-glucose medium and evolved gas actively. However, denitrification was not observed in thiosulfate medium supplemented with nitrate. *T. novellus* THI 031^T and *T. perometabolis* THI 023^T were obligate aerobes.

Both strains of *T. perometabolis* (THI 023^T and THI 024) were able to grow in a liquid medium supplemented with thiosulfate (5); we observed regular cell yields of ca. 30 to 40 µg of protein per ml and an optical density at 660 nm of ca. 0.1 during successive transfers in liquid medium supplemented with sodium thiosulfate (5 g/liter). This growth was accompanied by decreases in the pH of the medium. The cell yields in this medium corresponded to the yields obtained with other facultatively chemolithotrophic *Thiobacillus* strains, such as *Thiobacillus* sp. strain A2^T, *T. novellus* THI 031^T, *Thiobacillus intermedius* THI 101, and "*Thiobacillus deli-*

TABLE 2. Characteristics of *Thiobacillus* sp. strain A2^T, *T. perometabolis* THI 024, *T. novellus* THI 031^T, and *T. perometabolis* THI 023^T

Character	<i>Thiobacillus</i> sp. strain A2 ^T	<i>T. perometabolis</i> THI 024	<i>T. novellus</i> THI 031 ^{Ta}	<i>T. perometabolis</i> THI 023 ^T
Cell morphology	Rod	Rod	Rod	Rod
Cell width	0.6–0.7 µm	0.5–0.7 µm	0.7–0.8 µm	0.4–0.5 µm
Cell length	1.0–1.3 µm	1.0–1.5 µm	1.1–1.5 µm	1.1–1.7 µm
Motility	+	+	—	+
Flagellation	Tuft	Tuft	—	Polar
Spore formation	—	—	—	—
Gram reaction	—	—	—	—
Acid-alcohol-fast	—	—	—	—
Nitrate reduction ^b	+	+	—	—
Denitrification ^b	+	+	—	—
Denitrification ^c	—	—	—	—
Assimilation of:				
Elemental sulfur	w ^d	w	—	+
Thiocyanate	—	—	—	—
Thiosulfate	+	+	+	+
Tetrathionate	—	—	+	+
Nitrogen source ^e				
KNO ₃	+	+	+	+
NH ₄ Cl	+	+	+	+
Urea	—	—	+	+
Glutamate	+	+	+	+
Aspartate	+	+	—	+
Temperature for growth ^f				
Optimum	30–37°C	30–37°C	25–30°C	35–37°C
Maximum	40°C	42°C	37°C	42°C
Minimum	17°C	15°C	10°C	15°C
Initial pH for growth ^f				
Optimum	7.5–8.0	7.5–8.0	7.0	5.5–6.0
Maximum	9.5	9.5	7.5	7.0
Minimum	6.5	6.5	6.0	5.0
Initial pH for growth ^c				
Optimum	8.0–9.0	7.5–8.0	7.0–7.4	5.5–6.0
Maximum	9.5	9.5	9.0	7.5
Minimum	6.5	6.5	5.7	5.0
Hydrolysis of gelatin	—	—	—	—
Nutritional requirement	—	—	Biotin	Reduced inorganic sulfur compound
G + C content of deoxyribonucleic acid	67.0 mol%	67.1–67.8 mol%	67.3 mol%	65.0 mol%
Ubiquinone system ^g	Q-10	Q-10	Q-10	Q-8
Major cellular fatty acids ^h				
Non-hydroxylated	18:1 + 19cyc	18:1 + 19cyc	18:1 + 19cyc	16:0, 16:1 + 17cyc, 18:1 + 19cyc
Hydroxylated	3-OH 10:0	3-OH 10:0	ND ⁱ	3-OH 10:0, 3-OH 12:0

^a From reference 3.^b Determined in SM basal salts medium containing 5% (wt/vol) glucose, except for *T. perometabolis* THI 023^T, which was grown in SM basal salts medium containing 5% (wt/vol) Na₂S₂O₃ · 5H₂O and 0.4% (wt/vol) malate.^c Determined in SM basal salts medium containing 5% (wt/vol) Na₂S₂O₃ · 5H₂O.^d w, Weakly positive.^e Determined in SM basal salts medium containing 5% (wt/vol) Na₂S₂O₃ · 5H₂O and SM basal salts medium containing 5% (wt/vol) glucose, except for *T. perometabolis* THI 023^T, which was grown in SM basal salts medium containing 5% (wt/vol) Na₂S₂O₃ · 5H₂O and SM basal salts medium containing 5% (wt/vol) Na₂S₂O₃ · 5H₂O and 0.4% (wt/vol) malate.^f Determined in BY medium, except for *T. perometabolis* THI 023^T, which was grown in SM basal salts medium containing 5% (wt/vol) Na₂S₂O₃ · 5H₂O and 5% (wt/vol) yeast extract.^g Q-10, Ubiquinone 10; Q-8, ubiquinone 8.^h 18:1, Octadecenoic acid; 19cyc, cyclopropane acid of C₁₉; 16:0, hexadecanoic acid; 16:1, hexadecenoic acid; 17cyc, cyclopropane acid of C₁₇; 3-OH 10:0, 3-hydroxydecanoic acid; 3-OH 12:0, 3-hydroxydodecanoic acid.ⁱ ND, Not detected.

FIG. 1. Electron micrographs of *Thiobacillus* sp. strain A2^T (A), *T. perometabolis* THI 024 (B), and *T. perometabolis* THI 023^T (C), showing flagellum arrangement. The preparations were stained with phosphotungstic acid. Bar = 1 μ m.

catus'' THI 091, as determined in our laboratory. *Thiobacillus* sp. strain A2^T and *T. novellus* THI 031^T utilized thiosulfate for autotrophic growth and decreased the pH of the medium (13, 14).

Thiobacillus sp. strain A2^T and *T. perometa-*

bolis THI 024 were able to oxidize elemental sulfur weakly, as determined by changes in the pH of the medium; during three transfers the pH decreased by about 0.5 u. On the other hand, the pH of a medium inoculated with *T. novellus* THI 031^T, which did not utilize elemental sulfur, hardly changed; a similar result was observed with a non-inoculated system after 3 weeks of incubation.

Thiobacillus sp. strain A2^T and *T. perometabolis* THI 024 did not assimilate tetrathionate or thiocyanate. *T. novellus* THI 031^T used tetrathionate for autotrophic growth (the pH of the medium decreased) and did not use elemental sulfur, as described above (3). *T. perometabolis* THI 023^T oxidized elemental sulfur and tetrathionate in an autotrophic liquid medium, and the pH of the medium decreased after 1 week of incubation.

Thiobacillus sp. strain A2^T and *T. perometabolis* THI 024 used potassium nitrate and ammonium chloride as nitrogen sources in both SM basal salts-thiosulfate medium and SM basal salts-glucose medium, but they did not use urea. Potassium nitrate, ammonium chloride, and urea were used by *T. novellus* THI 031^T in SM basal salts-thiosulfate and SM basal salts-glucose media (3) and by *T. perometabolis* THI 023^T in SM basal salts-thiosulfate and SM basal salts-thiosulfate-malate media.

Thiobacillus sp. strain A2^T and *T. perometabolis* THI 024 grew well at pH 7.5 to 8.0 in BY medium, whereas *T. novellus* THI 031^T grew well at pH 7.0 (3). Slightly acidic conditions (pH 5.5 to 6.0 in SM basal salts-thiosulfate or SM basal salts-thiosulfate-yeast extract medium) produced the best growth of *T. perometabolis* THI 023^T.

Thiobacillus sp. strain A2^T and *T. perometabolis* THI 024 did not require any growth factor, whereas *T. novellus* THI 031^T required biotin (3, 9). *T. perometabolis* THI 023^T was able to grow in a defined medium supplemented with a single carbon source (Table 3) but only after a long lag period; the presence of thiosulfate with the carbon source markedly shortened the lag time. Thus, this organism seemed to require reduced sulfur compounds for optimal growth.

Assimilation of organic compounds. *Thiobacillus* sp. strain A2^T and *T. perometabolis* THI 024 assimilated amino acids, carbohydrates, organic acids, aromatic compounds, and alcohols, as shown in Table 3. *T. novellus* THI 031^T assimilated a more limited number of carbon compounds than *Thiobacillus* sp. strain A2^T and *T. perometabolis* THI 024 (3). The generation times of *Thiobacillus* sp. strain A2^T and *T. perometabolis* THI 024 were 2 and 2.2 h, respectively, in liquid SM basal salts medium supplemented with 20 mM glutamate. The generation time of *T.*

TABLE 3. Utilization of organic compounds by *Thiobacillus* sp. strain A2^T, *T. perometabolis* THI 024, *T. novellus* THI 031^T, and *T. perometabolis* THI 023^T

Substrate	<i>Thiobacillus</i> sp. strain A2 ^a	<i>T. perometabolis</i> THI 024 ^a	<i>T. novellus</i> THI 031 ^{Ta}	<i>T. perometabolis</i> THI 023 ^{Tb}
L-Alanine	+	+	+	+
L-Serine	+	+	w ^c	-
L-Leucine	+	+	-	-
L-Isoleucine	+	+	-	-
L-Aspartate	+	+	-	w
L-Glutamate	+	+	+	+
L-Proline	+	+	+	-
L-Histidine	+	+	+	-
L-Phenylalanine	+	+	w	-
L-Tryptophan	-	-	-	-
L-Cysteine	-	-	-	-
L-Arabinose	+	+	+	-
D-Ribose	+	+	+	-
D-Glucose	+	+	+	-
D-Galactose	+	+	+	-
Fructose	+	+	+	-
Lactose	-	-	-	-
Maltose	+	+	w	-
Sucrose	+	+	w	-
Gluconate	+	+	+	-
Glycerol	+	+	+	-
Mannitol	+	+	+	-
Cellobiose	-	-	-	-
Formate	+	+	+	-
Acetate	+	+	+	-
Propionate	+	+	w	-
n-Butyrate	+	+	-	-
Lactate	+	+	+	-
Pyruvate	+	+	+	-
L-Malate	+	+	+	w
Succinate	+	+	+	w
Citrate	-	-	+	w
α-Ketoglutarate	+	+	w	-
Glutarate	+	+	w	-
Oxalate	-	-	+	-
Adipate	+	+	-	-
Pimrate	-	-	-	-
Methanol	w	w	w	-
Ethanol	+	+	+	-
n-Propanol	+	+	+	-
n-Butanol	+	+	w	-
Cyclohexanol	-	-	-	-
Benzoate	+	+	-	-
o-Hydroxybenzoate	-	-	-	-
m-Hydroxybenzoate	+	+	-	-
p-Hydroxybenzoate	+	+	-	-
p-Aminobenzoate	-	-	-	-
DL-Mandelate	-	-	-	-

^a Determined on SM basal salts medium.

^b Determined on SM basal salts medium and on SM basal salts medium containing 5% (wt/vol) Na₂S₂O₃ · 5H₂O (results in parentheses).

^c w, Weakly positive.

novellus THI 031^T was 10 times longer (3) than the generation times of *Thiobacillus* sp. strain A2^T and *T. perometabolis* THI 024 in the same medium.

After a prolonged lag phase (about 2 weeks), *T. perometabolis* THI 023^T grew slowly in heterotrophic media containing alanine, glutamate, malate, citrate, succinate, or aspartate. This organism tended to form floc in liquid media containing these compounds. The maximum cell yields (expressed as optical densities at 660 nm) were 0.6 and 0.2 in SM basal salts medium containing 20 mM glutamate and SM basal salts medium containing 20 mM malate, respectively. The lag phase was shortened by adding thiosulfate to the media, as mentioned above. Furthermore, *T. perometabolis* THI 023^T grew in mixotrophic medium containing α-ketoglutarate, serine, or histidine. Weak growth, as determined by colony formation on plates and by optical density in liquid media, was observed in a mixotrophic medium containing glucose or gluconate.

Description of *Thiobacillus rapidicrescens* Katayama-Fujimura and Kuraishi sp. nov. In their morphological, physiological, and chemotaxonomic characteristics, *Thiobacillus* sp. strain A2^T and *T. perometabolis* THI 024 were quite similar to each other and distinct from the known *Thiobacillus* species. We propose a new species, *Thiobacillus rapidicrescens* (ra.pi.di.cres'cens. L. adj. *rapidus* rapid; L. part. adj. *crescens* growing; M. L. part. adj. *rapidicrescens* rapid growing), for *Thiobacillus* sp. strain A2^T and *T. perometabolis* THI 024; the description of this species is based on the characteristics of the type strain, strain A2 (= THI 041).

(i) **Cell morphology.** Rod-shaped cells (average dimensions, 0.4 to 0.5 μm wide by 1.1 to 1.7 μm long) with rounded ends. Cells occur singly or in pairs. Motile by means of tufts of polar flagella. Nonsporeforming. Gram negative. Not acid-alcohol-fast.

(ii) **Colonial appearance.** Colonies on BY medium are circular, wrinkled with raised centers, lobate, spreading, brownish yellow, opaque, and about 3 to 4 mm in diameter after 2 days of incubation. Colonies on thiosulfate medium are circular, convex, smooth, entire, creamy white, and opaque, developing a brown center with age; these colonies are about 2 mm in diameter after 1 week of incubation.

(iii) **Biochemical and physiological properties.** *T. rapidicrescens* is facultatively anaerobic and in heterotrophic medium supplemented with nitrate grows anaerobically and evolves gas actively.

This species is a facultative chemolithotroph. Thiosulfate is utilized for growth as an energy source. Elemental sulfur is oxidized slowly. Tet-

rathionate or thiocyanate does not support growth.

Potassium nitrate and ammonium chloride are utilized as nitrogen sources, but urea is not. Glutamate and aspartate are utilized as both nitrogen and carbon sources.

Alanine, serine, leucine, isoleucine, aspartate, glutamate, proline, histidine, phenylalanine, arabinose, ribose, glucose, galactose, fructose, maltose, sucrose, gluconate, glycerol, mannitol, formate, acetate, propionate, *n*-butyrate, lactate, pyruvate, malate, succinate, α -ketoglutarate, glutarate, adipate, ethanol, *n*-propanol, *n*-butanol, benzoate, *m*-hydroxybenzoate, and *p*-hydroxybenzoate are utilized as carbon sources. Heterotrophic growth is not stimulated by the presence of thiosulfate.

Growth occurs at 17 to 40°C, but not at 10 or 42°C; optimum growth occurs between 30 and 37°C.

Growth occurs at pH 6.5 to 9.5, and optimum growth occurs at pH 7.5 to 8.0 in BY medium and at pH 8.0 to 9.0 in SM basal salts-thiosulfate medium.

Gelatin is not liquefied.

Growth factor is not required.

G+C content: 67.0 mol%.

Ubiquinone system: ubiquinone 10.

This organism has octadecenoic acid plus cyclopropane acid of C₁₉ as major non-hydroxylated cellular fatty acids and 3-hydroxydecanoic acid as a hydroxylated fatty acid.

Isolated from soil (14).

The type strain of this organism is strain THI 041; this strain has been deposited with the American Type Culture Collection under accession number ATCC 25364. An additional strain, *T. rapidicrescens* THI 024, has been deposited with the American Type Culture Collection under accession number ATCC 27793 (1).

DISCUSSION

T. perometabolis has been regarded as a non-autotrophic organism on the basis of its inability to grow in a thiosulfate-mineral salts liquid medium when it is inoculated from colonies on plates containing medium of the same composition (8, 16). In our study, strains THI 023^T and THI 024 readily initiated growth in a liquid thiosulfate medium when they were transferred from cultures derived from successive inoculations on agar slants of the same composition, as well as agar media for mixotrophic growth. Furthermore, adequate growth was observed after transfer into a thiosulfate-mineral salts liquid medium. Therefore, we considered this organism to be able to grow autotrophically. *T. perometabolis* THI 023^T grew in heterotrophic media containing yeast extract or Casamino Acids (8) and single carbon compounds, such as

TABLE 4. Characteristics that differentiate *T. rapidicrescens* and *T. novellus*

Character	<i>T. rapidicrescens</i>	<i>T. novellus</i>
Motility	+	—
Nitrate reduction in heterotrophic medium	+	—
Denitrification in heterotrophic medium	+	—
Assimilation of tetrathionate	—	+
Initial pH of BY medium for optimum growth	7.5–8.0	7.0
Optimum temperature for growth	30–37°C	25–30°C
Generation time in BY medium	1 h	3 h
Nutritional requirement	—	Biotin
3-OH 10:0 fatty acid ^a	+	—

^a 3-OH 10:0, 3-Hydroxydecanoic acid.

malate, citrate, succinate, alanine, or aspartate. Growth in the media containing single carbon compounds required long lag periods. On the basis of these results, this organism was considered to be a facultative chemolithotroph, contrary to the original description (8). The name *T. perometabolis* appeared on the Approved Lists of Bacterial Names (11). Therefore, emendation of *T. perometabolis* is needed for exact identification of this species (4).

T. perometabolis THI 024 was able to grow on media containing a wide range of organic compounds in addition to thiosulfate, and this strain was also considered a facultative chemolithotroph. The pattern of assimilation of amino acids by *T. perometabolis* THI 024 was more versatile than the pattern described by Myers and Miller (10), who examined amino acids as both nitrogen and carbon sources.

Thiobacillus sp. strain A2^T was isolated from an anaerobic enrichment culture of "*Thiobacillus denitrificans*" and has been considered a strain or biovar of *T. novellus* (14). Characteristics useful for differentiating *Thiobacillus* sp. strain A2^T and *T. novellus* THI 031^T have been identified through our taxonomic studies of the genus *Thiobacillus* (3, 5). As a result, we have concluded that these two organisms belong to different taxa. Table 4 shows the characteristics that are useful for identifying *T. rapidicrescens*.

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