Physiological Characteristics of the Facultatively Chemolithotrophic *Thiobacillus* Species *Thiobacillus delicatus* nom. rev., emend., *Thiobacillus perometabolis*, and *Thiobacillus intermedius*

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The physiological characteristics of three facultatively chemolithotrophic *Thiobacillus* species with ubiquinone 8 (group II) (*Thiobacillus delicatus* THI 091^T [T = type strain] *Thiobacillus perometabolis* THI 023^T, and *Thiobacillus intermedius* THI 101^T) were compared. These organisms differ from other facultatively chemolithotrophic *Thiobacillus* strains with ubiquinone 10 (group I) in requiring thiosulfate or elemental sulfur for optimum growth. *T. delicatus* THI 091^T, *T. perometabolis* THI 023^T, and *T. intermedius* THI 101^T are regarded as three distinct species on the basis of their physiological characteristics. *T. delicatus* THI 091^T differs from *T. perometabolis* THI 023^T and *T. intermedius* THI 101^T in its lack of motility, its ability to grow anaerobically with nitrate, and its intermediate formation of polythionate during thiosulfate oxidation. *T. perometabolis* THI 023^T is distinguished from *T. intermedius* THI 101^T by its ability to grow heterotrophically with a single carbon source. *T. delicatus* is not on the Approved Lists of Bacterial Names; therefore, this name is revived. In addition, the description of this organism is emended, considering its inability to grow heterotrophically with a single carbon source.

Species of the genus *Thiobacillus* have been classified into three major groups and six subgroups according to their nutritional characteristics, the ubiquinone systems in their respiratory chains, their cellular fatty acid compositions, and the guanine-plus-cytosine contents of their deoxyribonucleic acids (DNAs) (8). DNA-DNA homology studies between the species of the genus *Thiobacillus* have supported this classification system (4).

In our classification system of *Thiobacillus* (8), the facultative chemolithotrophs with ubiquinone 10 comprise group I, and the organisms with ubiquinone 8 comprise group II, which includes *Thiobacillus delicatus* THI 091^T (T = type strain), *Thiobacillus perometabolis* THI 023^T, and *Thiobacillus intermedius* THI 101^T. *T. delicatus* was isolated by Mizoguchi et al. (14) from mine water, and its thiosulfate oxidation pathway has been investigated (18). *T. perometabolis* was isolated by London and Rittenberg (13) from soil. This organism was described as a chemolithotrophic heterotroph (17) which obtains energy from an inorganic compound and carbon from an organic substance. In contrast to this description, we found that *T. perometabolis* THI 023^T is able to grow autotrophically in mineral salts medium supplemented with thiosulfate, elemental sulfur, or tetrathionate (5), and we emended the description accordingly (7). London (12) isolated *T. intermedius* from soil and described it as being able to grow in autotrophic, mixotrophic, or complex heterotrophic medium.

As previously described (4), the indices of DNA-DNA homology among *T. delicatus* THI 091^T, *T. perometabolis* THI 023^T, and *T. intermedius* THI 101^T are low, and these organisms have been regarded as three distinct species. In this paper, we describe the physiological traits of these organisms relative to the taxonomy of the genus *Thiobacillus*. In addition, the name *T. delicatus*, which was not included on the Approved Lists of Bacterial Names (19), is revived. We also emend the description of *T. delicatus* THI 091^T regarding its inability to grow heterotrophically with a

single carbon source, in contrast to the characteristics given in the original description (14).

MATERIALS AND METHODS

Bacterial strains. *T. perometabolis* THI 023^T and *T. intermedius* THI 101^T (Table 1) were maintained on SM basal salts medium (8) (pH 6.0) supplemented with 0.5% Na₂S₂O₃ · 5H₂O and 0.5% yeast extract (Difco Laboratories, Detroit, Mich.); *T. delicatus* THI 091^T was maintained on SM basal salts medium containing 0.5% Na₂S₂O₃ · 5H₂O and 0.1% yeast extract. The strains were transferred once a week.

Identification. Physiological characteristics were examined in SM basal salts medium (pH 6.0) containing various supplements, as described previously (5, 6). 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 (24), and 1,000 ml of deionized water.

Nitrate respiration was examined anaerobically in a mixotrophic medium supplemented with 0.5% Na₂S₂O₃ · 5H₂O and 0.4% sodium malate and in an autotrophic medium containing 0.5% Na₂S₂O₃ · 5H₂O, as described previously (6). The tests for *T. delicatus* THI 091^T were also done in a medium containing 0.3% K₂S₄O₆ or 0.38% Na₂S₃O₆ · 3H₂O.

Assimilation of organic compounds was examined by using both heterotrophic and mixotrophic SM medium agar plates (pH 6.0) supplemented with various substrates at concentrations of 5 to 100 mM (21), as previously described (5, 6). The mixotrophic media contained 0.5% Na₂S₂O₃ · 5H₂O in addition to the organic compounds. 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 the colonies on a control plate which contained only the basal salts medium (5). When a positive response was observed on a mixotrophic agar medium, the growth was confirmed in liquid medium of the same composition. These experiments produced two types of growth effects. One was significant growth stimulation, and this was designated a positive reaction. The other was weak growth without stimulation, and the growth yield

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TABLE 1. Bacterial strains used^a

Strain	Species	Source	Other designation	Reference(s)
THI 091 ^T	<i>T. delicatus</i>	T. Mizoguchi	IAM 12624 ^T	14
THI 023 ^T	<i>T. perometabolis</i>	IAM 12132 ^T	ATCC 23370 ^T	5, 7, 13
THI 101 ^T	<i>T. intermedius</i>	IAM 12131 ^T	ATCC 15466 ^T	12

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

was similar to that on thiosulfate medium; such a reaction was designated a weak positive reaction.

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), as described previously (5). Thiosulfate and tetrathionate were autoclaved separately, and trithionate was sterilized by filtration and added to the basal salts medium. The powdered sulfur which we used was steamed intermittently.

Growth experiments. Liquid cultures were incubated in 500-ml culture flasks containing 100 ml of SM basal salts medium (pH 6.0) supplemented with a sulfur compound or an organic compound. A small number of cells was inoculated into the medium to avoid carrying over materials; accord-

ingly, one loopful of cells from the maintenance culture was suspended in 10 ml of sterilized water, and 1 ml of the suspension was inoculated into 100 ml of fresh medium. Growth was monitored by measuring the absorbance at 660 nm. During incubation at 30°C with reciprocal shaking, the pH of the medium was maintained at pH 6.0 to 6.5 with 5% Na₂CO₃ or 1 N H₂SO₄.

The effects of organic sulfur compounds, thiamine pyrophosphate, reduced glutathione, lipoic acid, biotin, coenzyme A, methionine, and cysteine on the growth of *T. intermedius* THI 101^T were analyzed in MS mineral salts medium supplemented with a trace metal solution (1), vitamin B₁₂ (0.1 mg/liter), and glucose (0.5%) as described by Smith and Rittenberg (20), except that bromothymol blue was not present and smaller inocula were used with mixotrophically grown cells, as described above. The cultures were left stationary for 3 days at 30°C after inoculation and were then placed on a reciprocal shaker (20).

Analytical methods. Tetrathionate, trithionate, and thiosulfate were analyzed by the cyanolysis method (9). Sulfate was determined gravimetrically after the addition of 5% BaCl₂ · 2H₂O in 0.1 N HCl. Glutamate and aspartate were measured by the ninhydrin reaction, and glucose was measured by the glucose oxidase-peroxidase method.

Chemicals. Sodium trithionate was kindly supplied by M. Ishimoto, Faculty of Pharmaceutical Science, Hokkaido University, Sapporo, Japan. Potassium tetrathionate was prepared as described by Trudinger (22). Coenzyme A was obtained from the Oriental Yeast Co. Ltd., Tokyo, Japan.

TABLE 2. Characteristics of *T. delicatus* THI 091^T, *T. perometabolis* THI 023^T, and *T. intermedius* THI 101^{Ta}

Character	<i>T. delicatus</i> THI 091 ^T	<i>T. perometabolis</i> THI 023 ^{Tb}	<i>T. intermedius</i> THI 101 ^T
Cell width (μm)	0.4–0.6	0.4–0.5	0.6–0.8
Cell length (μm)	1.0–1.6	1.1–1.7	1.0–1.4
Motility	—	+	+
Flagellation	—	Polar	Polar
Nitrate reduction ^{c,d}	+	—	—
Assimilation of:			
Elemental sulfur	+	+	+
Thiocyanate	—	—	—
Thiosulfate	+	+	+
Tetrathionate	+	+	+
Temp for growth (°C) ^e			
Optimum	30–35	35–37	30–35
Maximum	42	42	37
Minimum	15	15	15
Initial pH for growth ^e			
Optimum	5.5–6.0 (5.5–6.0)	5.5–6.0 (5.5–6.0)	5.5–6.0 (5.5–6.0)
Maximum	7.0 (7.0)	7.0 (7.5)	7.5 (7.0)
Minimum	5.0 (5.0)	5.0 (5.0)	5.0 (5.0)
Nutritional requirement	Reduced inorganic sulfur compound	Reduced inorganic sulfur compound	Reduced inorganic sulfur compound
Growth with a single carbon source	—	+	—
Guanine-plus-cytosine content of DNA (mol%) ^f	66.0	65.0	64.9
Hydroxylated cellular fatty acids ^{f,g}	3-OH 10:0, 3-OH i10:0	3-OH 10:0, 3-OH 12:0, 3-OH i10:0	3-OH 10:0, 3-OH 12:0

^a In addition, negative responses were observed with all three species for the following characters: Gram reaction, spore formation, acid-alcohol fastness, denitrification in autotrophic and mixotrophic media, and gelatin hydrolysis. Potassium nitrate, ammonium chloride, urea, glutamate, and aspartate were used as nitrogen sources. The cells of these organisms are rod shaped, contain ubiquinone 8 in their respiratory chains, and contain hexadecanoic acid, hexadecenoic acid plus C₁₇ cyclopropane acid, and octadecenoic acid plus C₁₉ cyclopropane acid as their major non-hydroxylated cellular fatty acids.

^b Data from reference 5.

^c Determined in SM basal salts medium containing 0.5% Na₂S₂O₃ · 5H₂O and 0.4% sodium malate.

^d Determined in SM basal salts medium containing 0.5% Na₂S₂O₃ · 5H₂O.

^e Determined in SM basal salts medium containing 0.5% Na₂S₂O₃ · 5H₂O and 0.5% yeast extract and in SM basal salts medium containing 0.5% Na₂S₂O₃ · 5H₂O (in parentheses).

^f Data from reference 8.

^g 3-OH 10:0, 3-Hydroxydecanoic acid; 3-OH i10:0, 3-hydroxy-8-methylnonanoic acid; 3-OH 12:0, 3-hydroxydodecanoic acid.

RESULTS

Morphological and physiological characteristics. Table 2 shows the morphological and physiological characteristics of *T. delicatus* THI 091^T, *T. perometabolis* THI 023^T (5), and *T. intermedius* THI 101^T.

T. delicatus THI 091^T was able to use nitrate for respiration on thiosulfate, trithionate, or tetrathionate in autotrophic or mixotrophic medium containing sodium malate. About 10 and 50% of the sulfur moiety of the trithionate added initially were recovered as thiosulfate after 1 day and 1 week of incubation, respectively, in a system which was not inoculated with cells. *T. delicatus* THI 091^T did not evolve gas and accumulated nitrite in the medium. *T. perometabolis* THI 023^T (13) and *T. intermedius* THI 101^T were obligate aerobes.

T. delicatus THI 091^T, *T. perometabolis* THI 023^T, and *T. intermedius* THI 101^T grew autotrophically in SM basal salts medium supplemented with elemental sulfur, thiosulfate, or tetrathionate; this growth was accompanied by a decrease in the pH of the medium. The pH of the thiosulfate medium inoculated with *T. delicatus* THI 091^T transiently increased due to formation of tetrathionate. Thiocyanate did not support the growth of these organisms after 3 weeks of incubation.

Assimilation of organic compounds. *T. delicatus* THI 091^T, *T. perometabolis* THI 023^T, and *T. intermedius* THI 101^T

TABLE 3. Growth of *T. delicatus* THI 091^T, *T. perometabolis* THI 023^T, and *T. intermedius* THI 101^T with various carbon sources in mixotrophic medium supplemented with 0.5% Na₂S₂O₃ · 5H₂O^a

Substrate	Concn (mM)	Growth of:		
		<i>T. delicatus</i> THI 091 ^T	<i>T. perometabolis</i> THI 023 ^{Tb}	<i>T. intermedius</i> THI 101 ^T
L-Alanine	20	+	+	+
L-Serine	40	—	+	+
L-Aspartate	20	+	+	+
L-Glutamate	20	+	+	+
L-Proline	20	w ^c	—	—
L-Histidine	5	w	w	w
D-Glucose	20	w	w	+
Fructose	20	w	—	+
Gluconate	20	w	w	w
Lactate	20	w	—	+
L-Malate	20	+	+	+
Succinate	20	+	+	+
Citrate	20	+	+	+
α-Ketoglutarate	20	+	+	+
Oxalate	20	w	—	w
Ethanol	20	w	w	w
n-Propanol	20	w	—	—

^a Growth after 2 weeks of incubation was determined as described in the text. In addition, the three species showed negative responses with the following compounds: L-leucine (20 mM), L-isoleucine (20 mM), L-phenylalanine (10 mM), L-tryptophan (5 mM), L-cysteine (20 mM), L-arabinose (20 mM), D-ribose (20 mM), D-galactose (20 mM), lactose (20 mM), maltose (20 mM), sucrose (20 mM), glycerol (20 mM), mannitol (20 mM), cellobiose (20 mM), formate (100 mM), acetate (20 mM), propionate (20 mM), n-butyrate (20 mM), pyruvate (20 mM), glutarate (20 mM), adipate (20 mM), pimelate (20 mM), methanol (20 mM), n-butanol (20 mM), cyclonexanol (10 mM), benzoate (10 mM), o-hydroxybenzoate (10 mM), m-hydroxybenzoate (10 mM), p-hydroxybenzoate (10 mM), p-aminobenzoate (10 mM), and DL-mandelate (10 mM).

^b Data from reference 5.

^c w, Weakly positive.

assimilated alanine, aspartate, glutamate, malate, succinate, citrate, and α-ketoglutarate in the mixotrophic medium supplemented with 0.5% Na₂S₂O₃ · 5H₂O (Table 3). Serine, glucose, fructose, and lactate were also used by *T. perometabolis* THI 023^T and *T. intermedius* THI 101^T. In general, these group II organisms did not grow by using organic compounds in the absence of thiosulfate (data not shown); an exception was *T. perometabolis* THI 023^T, which grew heterotrophically after a long lag period in medium containing alanine, glutamate, aspartate, malate, succinate, or citrate, as reported previously (5).

Smith and Rittenberg (20) reported that *T. intermedius* requires organic sulfur compounds for growth in the absence of thiosulfate. We repeated the experiments of these authors as carefully as possible according to their report to confirm this requirement. The cell yield in basal salts medium containing 0.5% glucose and 0.5% yeast extract was about 0.3U after 1 week of incubation, as determined by optical density at 660 nm. On the other hand, the yield in basal salts-glucose medium supplemented with organic sulfur compounds was 0.02U, from which was almost the same as the yield in basal salts-glucose medium (optical density at 660 nm, 0.03U); thus, organic sulfur compounds had no effect on the growth of *T. intermedius* in our experiments.

Growth experiments in unithrophic and mixotrophic media. *T. delicatus* THI 091^T (Fig. 1) and *T. intermedius* THI 101^T (Fig. 2) did not grow in heterotrophic medium containing a single carbon source. On the other hand, *T. perometabolis* THI 023^T grew heterotrophically in medium containing a single carbon source after a lag period (Fig. 3). This organism grew without a lag period when the cells were inoculated into a medium of the same composition. *T. perometabolis* THI 023^T showed diauxic growth in mixotrophic medium containing 0.2% sodium glutamate, and the cells aggregated between the first growth and the second growth (Fig. 3). A second growth was not observed in mixotrophic medium containing 0.5% sodium glutamate (data not shown). *T. delicatus* THI 091^T oxidized thiosulfate to sulfate via intermediate formation of tetrathionate and then trithionate in both autotrophic and mixotrophic media (Fig. 1b and c). On the other hand, little or no tetrathionate or trithionate was detected in these culture systems with *T. intermedius* THI 101^T or *T. perometabolis* THI 023^T (Fig. 2b and 3b). The sulfur moiety was not recovered completely as sulfate in the media with *T. intermedius* THI 101^T. This may depend on the formation of elemental sulfur during thiosulfate oxidation. The growth of these organisms was stimulated in mixotrophic medium supplemented with thiosulfate. Elemental sulfur also stimulated the growth of *T. delicatus* THI 091^T (14), *T. perometabolis* THI 023^T, and *T. intermedius* THI 101^T; however, little or no effect was observed when tetrathionate was added. Under well-defined chemostat culture conditions *Thiobacillus novellus* benefits from a mixotrophic medium (10). However, under batch culture conditions the growth of group I species (e.g., *T. novellus* and *Thiobacillus versutus* [synonym, "*Thiobacillus rapidicrescens*"]) is not stimulated in mixotrophic medium (5, 6, 16). Therefore, the group II species may be differentiated from the group I species by a requirement for an inorganic sulfur compound for optimum growth in batch cultures, as well as by their chemotaxonomic traits, as described previously (8).

The mixotrophic growth of *T. perometabolis* THI 023^T was greatly influenced by the concentration of glutamate, as described above (Fig. 3). The concentration of yeast extract markedly affected the growth rates and cell yields of group II species in the mixotrophic medium supplemented with thio-

FIG. 1. Growth and thiosulfate oxidation by *T. delicatus* THI 091^T in unitrophic and mixotrophic media. (a) Growth of *T. delicatus* THI 091^T (solid lines) and concentration of aspartate (dashed lines). Symbols: ○, aspartate-thiosulfate medium; ●, thiosulfate medium; □, aspartate medium. (b and c) Concentration of sulfur compounds in aspartate-thiosulfate medium (b) and thiosulfate medium (c). Symbols: ■, $S_2O_3^{2-}$; △, $S_4O_6^{2-}$; ▲, $S_3O_6^{2-}$; ▽, SO_4^{2-} .

sulfate (Table 4). The highest growth rate and cell yield were found in media containing 0.3 to 0.5% yeast extract, and the higher concentrations caused diauxic growth, followed by a long lag period of about 5 to 10 days and inhibition of growth. These data suggest that group II species are more sensitive to organic compounds than group I species, whose growth is not inhibited by the presence of 1 to 2% yeast extract or other organic substances (6; unpublished data).

T. delicatus THI 091^T, *T. perometabolis* THI 023^T, and *T. intermedius* THI 101^T were regarded as three distinct species on the basis of our taxonomic studies of *Thiobacillus* species. *T. delicatus* THI 091^T differed from *T. perometabolis* THI 023^T and *T. intermedius* THI 101^T by the absence of 3-hydroxydodecanoic acid as a hydroxy cellular fatty acid (8), its lack of motility, its ability to grow anaerobically by using nitrate, and its thiosulfate oxidation pathway. *T. perometabolis* THI 023^T was differentiated from *T. intermedius* THI 101^T by its ability to grow heterotrophically with a single

carbon source. These results have been supported by DNA-DNA homology studies (4).

T. delicatus THI 091^T was described and named by Mizoguchi et al. (14). However, this name is not on the Approved Lists of Bacterial Names (19); it is revived in this paper. Mizoguchi et al. (14) reported weak growth of *T. delicatus* in mineral salts medium containing aspartate or pyruvate and indicated that this organism is able to grow heterotrophically with a single carbon source. However, we found that no heterotrophic growth occurred in a defined medium supplemented with aspartate (Fig. 1a), pyruvate, or other organic substance. The mode of energy generation is of great significance in the classification and identification of the species belonging to the genus *Thiobacillus* (8, 23). Therefore, we emend the description of *T. delicatus* with regard to heterotrophic growth and add new characters, as determined in this study.

Description of *Thiobacillus delicatus* Mizoguchi et al. 1976 nom. rev. Katayama-Fujimura and Kuraishi emend. Katayama-Fujimura and Kuraishi. The emended description of the biochemical and physiological characteristics of *T. delicatus* (*del.i.ca'tus*. L. masc. adj. *delicatus* delicate) is based on the characteristics of strain THI 091^T.

T. delicatus is a facultative anaerobe; it grows anaerobically in the presence of nitrate and accumulates nitrite in mixotrophic and autotrophic media supplemented with thiosulfate or tetrathionate.

FIG. 2. Growth and thiosulfate oxidation by *T. intermedius* THI 101^T in unitrophic and mixotrophic media. (a) Growth of *T. intermedius* THI 101^T. (b) Concentration of sulfur compounds (solid lines) and glucose (dashed lines). Symbols: ○, glucose-thiosulfate medium; ●, thiosulfate medium; □, glucose medium.

FIG. 3. Growth and thiosulfate oxidation by *T. perometabolis* THI 023^T in unithiophic and mixotrophic media. (a) Growth of *T. perometabolis* THI 023^T. Cells aggregated between the first growth and the second growth in glutamate-thiosulfate medium, and the growth could not be determined by optical density (dashed line). (b) Concentration of sulfur compounds (solid lines) and glutamate (dashed lines). Symbols: ○, glutamate-thiosulfate medium; ●, thiosulfate medium; □, glutamate medium.

This species is a facultative chemolithotroph. Heterotrophic growth occurs in a mixotrophic medium, but not with a single carbon source. In mixotrophic medium supplemented with thiosulfate, this species grows in the presence of alanine, aspartate, glutamate, malate, succinate, citrate, or α-ketoglutarate. It grows in liquid mineral salts medium supplemented with elemental sulfur, thiosulfate, or tetrathionate; this growth is accompanied by a decrease in the pH of the medium. Thiocyanate does not support growth.

Potassium nitrate, ammonium chloride, and urea are utilized as nitrogen sources. Glutamate and aspartate are utilized as both nitrogen and carbon sources.

Growth occurs at 15 to 42°C, but not at 10 or 45°C; optimum growth occurs between 30 and 35°C.

Growth occurs at pH 5.0 to 7.0, and optimum growth occurs at pH 5.5 to 6.0 in SM basal salts-thiosulfate-yeast extract medium and SM basal salts-thiosulfate medium.

Gelatin is not liquefied.

This species requires elemental sulfur or thiosulfate for optimum growth.

The guanine-plus-cytosine content is 66.0 mol%.

The ubiquinone system is ubiquinone 8.

This organism contains hexadecanoic acid, hexadecenoic acid plus C₁₇ cyclopropane acid, and octadecenoic acid plus C₁₉ cyclopropane acid as major nonhydroxy cellular fatty

TABLE 4. Effects of yeast extract concentration on the growth of *T. delicatus* THI 091^T, *T. perometabolis* THI 023^T, and *T. intermedius* THI 101^T

Yeast extract concn (g/liter)	Maximum cell yield ^a		
	<i>T. delicatus</i> THI 091 ^T	<i>T. perometabolis</i> THI 023 ^T	<i>T. intermedius</i> THI 101 ^T
0	0.18 (8) ^b	0.18 (7)	0.10 (4)
0.5	0.42 (4)	0.39 (2)	0.34 (3)
1.0	0.68 (4)	0.58 (2)	0.66 (3)
3.0	0.78 (5)	1.21 (2)	1.23 (3)
5.0	1.11 (13) ^c	1.61 (2)	1.15 (7) ^d
10.0	0.07 (13) ^c	1.33 (10) ^d	0.15 (7) ^c
15.0	0.10 (13) ^c	0.03 (12) ^c	ND ^e

^a Data are expressed as units of optical density at 660 nm. The medium used for *T. delicatus* THI 091^T and *T. intermedius* THI 101^T contained 5 g of Na₂S₂O₃ · 5H₂O per liter; the medium used for *T. perometabolis* THI 023^T contained 10 g of this compound per liter.

^b The numbers in parentheses are the cultivation times (in days) when maximum growth yield was determined.

^c Growth was accompanied by a lag period.

^d Growth curve was diauxic.

^e ND, Not determined.

acids, and 3-hydroxydecanoic acid and a trace amount of 3-hydroxy-8-methylnonanoic acid as hydroxylated fatty acids.

Isolated from mine water (14).

The type strain of this organism is strain THI 091; this strain has been deposited with the Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan, as strain IAM 12624.

DISCUSSION

Facultatively chemolithotrophic *Thiobacillus* strains are metabolically versatile and are able to grow autotrophically on elemental sulfur or inorganic and reduced sulfur compounds and heterotrophically on a variety of organic substances (21). However, utilization of organic substances by *T. delicatus* THI 091^T, *T. perometabolis* THI 023^T, and *T. intermedius* THI 101^T is limited to intermediates of the tricarboxylic acid cycle and amino acids which are closely linked with this cycle biosynthetically. Both organic substances and inorganic sulfur compounds, such as thiosulfate or elemental sulfur, are required for optimum growth. These physiological characteristics of group II species corroborate the classification of the species based on chemotaxonomic traits such as the ubiquinone system, cellular fatty acid composition, and guanine-plus-cytosine content of the DNA (8).

Previously (4), we found low levels of DNA-DNA homology between *T. perometabolis* THI 023^T and *T. intermedius* THI 101^T. Harrison (3) reported a close relationship between these organisms based on his DNA-DNA homology studies and concluded that these organisms are related at the subspecies level. This discrepancy may be due to differences in the experimental methods employed. We regard *T. perometabolis* THI 023^T and *T. intermedius* THI 101^T as separate species based on our DNA-DNA homology indices (4) among strains in all of the six subgroups previously proposed (8), together with their physiological (5, 7; this paper) and chemotaxonomic (8) characteristics.

Thiosulfate either is oxidized to tetrathionate by a thiosulfate-oxidizing enzyme (22) or is cleaved to sulfur and sulfide by a thiosulfate-cleaving enzyme (rhodanese), followed by the oxidation of sulfur and sulfide (2) by *Thiobacillus* species. The variation in intermediates obtained by different

workers has been interpreted as being due to growth conditions (15). Although slight transient formation of tetrathionate was sometimes observed in our experiments, this compound was not the end product of thiosulfate oxidation by *T. perometabolis* THI 023^T, by *T. intermedius* THI 101^T, by the group I-1 organisms *T. versutus* (synonym, "*T. rapidicrescens*") THI 041^T and THI 024 (5), or by *T. novellus* THI 031^T (6). *T. delicatus* THI 091^T was the only organism to produce considerable amounts of tetrathionate and then trithionate as intermediate metabolites of thiosulfate oxidation to sulfate, as far as we have determined.

We have previously described the autotrophic growth ability of *T. perometabolis* THI023^T in mineral salts medium supplemented with elemental sulfur, thiosulfate, or tetrathionate (5), and the description of this organism was emended previously (7). Recently, Harrison also reported the potential for autotrophic growth of *T. perometabolis* in thiosulfate medium (3).

Smith and Rittenberg (20) reported that *T. intermedius* lacks the ability to assimilate sulfate for its cellular materials and requires organic sulfur compounds for growth in the absence of thiosulfate. *Chlorobium thiosulfatophilum* has also been reported to grow in the presence of cysteine instead of thiosulfate (11). However, the growth of these organisms in medium supplemented with organic sulfur compound(s) has been reported to be limited (11, 20) compared with growth in medium containing thiosulfate. We could not confirm the requirement for organic sulfur compounds by using a culture system similar to the one described by Smith and Rittenberg (20). This might be because our small inoculum size (to prevent carrying over preculture materials) was too small for this organism to begin growth under such stressful conditions. Further study will be needed to understand in detail the significance of the requirement for inorganic sulfur compounds for the growth of organisms that oxidize sulfur compounds.

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LITERATURE CITED

- Allen, M. B. 1959. Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte. Arch. Mikrobiol. 32:270-277.
- Charles, A. M., and I. Suzuki. 1966. Mechanism of thiosulfate oxidation by *Thiobacillus novellus*. Biochim. Biophys. Acta 128:510-521.
- Harrison, A. P., Jr. 1983. Genomic and physiological comparisons between heterotrophic thiobacilli and *Acidiphilium cryptum*, *Thiobacillus versutus* sp. nov., and *Thiobacillus acidophilus* nom. rev. Int. J. Syst. Bacteriol. 33:211-217.
- Katayama-Fujimura, Y., Y. Enokizono, and H. Kuraishi. 1983. Deoxyribonucleic acid homologies among species of the genus *Thiobacillus*. J. Gen. Appl. Microbiol. 29:287-295.
- Katayama-Fujimura, Y., I. Kawashima, N. Tsuzaki, and H. Kuraishi. 1983. Reidentification of *Thiobacillus perometabolis* ATCC 27793 and *Thiobacillus* sp. strain A2 with reference to a new species, *Thiobacillus rapidicrescens* sp. nov. Int. J. Syst. Bacteriol. 33:532-538.
- Katayama-Fujimura, Y., and H. Kuraishi. 1980. Characterization of *Thiobacillus novellus* and its thiosulfate oxidation. J. Gen. Appl. Microbiol. 26:357-367.
- Katayama-Fujimura, Y., and H. Kuraishi. 1983. Emendation of *Thiobacillus perometabolis* London and Rittenberg 1967. Int. J. Syst. Bacteriol. 33:650-651.
- Katayama-Fujimura, Y., N. Tsuzaki, and H. Kuraishi. 1982. Ubiquinone, fatty acid and DNA base composition determination as a guide to the taxonomy of the genus *Thiobacillus*. J. Gen. Microbiol. 128:1599-1611.
- Kelly, D. P., L. A. Chambers, and P. A. Trudinger. 1969. Cyanolysis and spectrophotometric estimation of trithionate in mixture with thiosulfate and tetrathionate. Anal. Chem. 41:898-901.
- Leefeldt, R. H., and A. Matin. 1980. Growth and physiology of *Thiobacillus novellus* under nutrient-limited mixotrophic conditions. J. Bacteriol. 142:645-650.
- Lippert, K.-D., and N. Pfennig. 1969. Die Verwertung von molekularem Wasserstoff durch *Chlorobium thiosulfatophilum*. Wachstum und CO₂-Fixierung. Arch. Mikrobiol. 65:29-47.
- London, J. 1963. *Thiobacillus intermedius* nov. sp. A novel type of facultative autotroph. Arch. Mikrobiol. 46:329-337.
- London, J., and S. C. Rittenberg. 1967. *Thiobacillus perometabolis* nov. sp., a non-autotrophic thiobacillus. Arch. Mikrobiol. 59:218-225.
- Mizoguchi, T., T. Sato, and T. Okabe. 1976. New sulfur-oxidizing bacteria capable of growing heterotrophically, *Thiobacillus rubellus* nov. sp. and *Thiobacillus delicatus* nov. sp. J. Ferment. Technol. 54:181-191.
- Peck, H. D., Jr. 1968. Energy-coupling mechanisms in chemolithotrophic bacteria. Annu. Rev. Microbiol. 22:489-518.
- Perez, R. C., and A. Matin. 1980. Growth of *Thiobacillus novellus* on mixed substrates (mixotrophic growth). J. Bacteriol. 142:633-638.
- Rittenberg, S. C. 1969. The roles of exogenous organic matter in the physiology of chemolithotrophic bacteria. Adv. Microb. Physiol. 3:159-196.
- Sato, T., T. Mizoguchi, and T. Okabe. 1976. Oxidation of inorganic sulfur compounds by thiobacilli. J. Ferment. Technol. 54:361-368.
- Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.). 1980. Approved lists of bacterial names. Int. J. Syst. Bacteriol. 30:225-420.
- Smith, D. W., and S. C. Rittenberg. 1974. On the sulfur-source requirement for growth of *Thiobacillus intermedius*. Arch. Microbiol. 100:65-71.
- Taylor, B. F., and D. S. Hoare. 1969. New facultative *Thiobacillus* and a reevaluation of the heterotrophic potential of *Thiobacillus novellus*. J. Bacteriol. 100:487-497.
- Trudinger, P. A. 1961. Thiosulphate oxidation and cytochromes in *Thiobacillus* X. II. Thiosulphate-oxidizing enzyme. Biochem. J. 78:680-686.
- Vishniac, W. V. 1974. Genus *Thiobacillus*, p. 456-461. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Vishniac, W., and M. Santer. 1957. The thiobacilli. Bacteriol. Rev. 21:195-213.