Ubiquinone, Fatty acid and DNA Base Composition Determination as a Guide to the Taxonomy of the Genus *Thiobacillus*

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(Received 26 August 1981; revised 15 November 1981)

Facultatively chemolithotrophic thiobacilli, *Thiobacillus perometabolis* THI 022, *Thiobacillus* sp. A2, *Thiobacillus novellus*, *Thiobacillus organoparus* and *Thiobacillus acidophilus*, had ubiquinones with 10 isoprene units (Q-10) (group I). These species also had [octadecenoic acid (18:1) + cyclopropane acid of C₁₉ (19_{cyc})] as major non-hydroxylated fatty acids. The organisms, except *T. novellus*, contained 3-hydroxydecanoic acid (3-OH 10:0) or 3-hydroxytetradecanoic acid (3-OH 14:0) as hydroxy fatty acids. DNA base compositions of these species were 63 to 68 mol% G + C. Other facultatively chemolithotrophic thiobacilli, including *T. perometabolis* THI 023, *Thiobacillus delicatus* and *Thiobacillus intermedius* contained Q-8 (group II). They had hexadecanoic acid (16:0), [hexadecenoic acid (16:1) + cyclopropane acid of C₁₇ (17_{cyc})] and [18:1 + 19_{cyc}] as major non-hydroxylated fatty acids. The species of group II had 3-OH 10:0 and some possessed 3-hydroxydodecanoic acid (3-OH 12:0). The DNA base composition of these strains was about 65 mol% G + C. Obligate chemolithotrophic thiobacilli contained Q-8 (group III). They had 16:0, [16:1 + 17_{cyc}] with or without [18:1 + 19_{cyc}] as major non-hydroxylated fatty acids. The species of group III contained 3-OH 12:0 and some had 3-OH 10:0 when grown at neutral pH and 3-OH 14:0 in acidophilic conditions. DNA base compositions of these organisms ranged from 51 to 64 mol% G + C. Chemotaxonomic characteristics would seem to be useful for the identification and the classification of thiobacilli.

INTRODUCTION

Thiobacilli are Gram-negative rods, motile or non-motile, and obtain energy by the oxidation of reduced or partially reduced sulphur compounds. These organisms show various nutritional characteristics in the utilization of inorganic and organic substrates, which suggest that the genus *Thiobacillus* is heterogeneous.

Following several taxonomic studies of thiobacilli (Hutchinson *et al.*, 1965, 1966, 1967, 1969; Jackson *et al.*, 1968) new strains, which had heterotrophic or facultatively autotrophic characteristics, were isolated, i.e. *Thiobacillus perometabolis* (London & Rittenberg, 1967; Myers & Millar, 1975), *Thiobacillus* sp. A2 (Taylor & Hoare, 1969), *Thiobacillus delicatus* (Mizoguchi *et al.*, 1976), *Thiobacillus rubellus* (Mizoguchi *et al.*, 1976), *Thiobacillus organoparus* (Markosyan, 1973) and *Thiobacillus acidophilus* (Guay & Silver, 1974). The taxonomy of these species has not been reported in detail.

Cellular fatty acid (Agate & Vishniac, 1973) and DNA base composition (Jackson *et al.*, 1968) were used for the classification of the genus in *Bergey's Manual of Determinative Bacteriology*, 8th edition (Vishniac, 1974). In our previous papers (Katayama & Kuraishi, 1978; Katayama-Fujimura & Kuraishi, 1980), we pointed out misidentification of the cellular fatty acid compositions of *Thiobacillus thioparus* and *Thiobacillus novellus* as described by

Agate & Vishniac (1973). Considering these facts, a new taxonomic approach would be valuable for a better understanding of this genus. This paper deals with the quinone system, the cellular fatty acid composition and the DNA base composition of thiobacilli, and discusses the taxonomy of the genus *Thiobacillus*.

METHODS

Strains studied and growth conditions. The strains and their sources are listed in Table 1. *Thiobacillus thioparus* THI 112 was isolated in our laboratory as a thiocyanate-assimilating bacterium (Katayama & Kuraishi, 1978). Bacteria were cultured in heterotrophic, mixotrophic or autotrophic medium according to the physiological characteristics described by the original authors. Chemoorganotrophs and several facultative chemolithotrophs were grown in heterotrophic medium. Accordingly, *Thiobacillus trautweinii* THI 081 and THI 082 and *Thiobacillus* sp. A2 THI 041 were grown in BY medium (Katayama-Fujimura & Kuraishi, 1980). *Thiobacillus organoparus* THI 071 was grown in a medium described by Markosyan (1973) supplemented with 0.5% (w/v) glucose. *Thiobacillus acidophilus* THI 061 was grown in 9K medium (Silverman & Lundgren, 1959) supplemented with 1% (w/v) glucose as described by Guay & Silver (1974). For the preparation of DNA, *T. rubellus* THI 001 was grown in mixotrophic medium [(%, w/v): meat extract, 0.5; peptone (Kyokuto, Tokyo, Japan), 1; $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, 0.5; pH 6.5; Mizoguchi *et al.* 1976]. For the extraction of quinone and cellular fatty acids, the organism was cultured in SMM medium supplemented with 0.5% (w/v) glucose and 0.1% (w/v) yeast extract (Kyokuto) because a prolonged time lag was observed in the mixotrophic medium. SMM medium contained (%, w/v): KH_2PO_4 , 0.245; Na_2HPO_4 , 0.1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01; NH_4Cl , 0.03; 0.25 ml per 100 ml of a trace metal solution (Vishniac & Santer, 1957); pH 6.5.

Thiobacillus perometabolis THI 022 was cultured in medium 152 (Gherna & Pienta, 1980) with 0.5% (w/v) $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ and 1% (w/v) yeast extract (Oriental, Tokyo, Japan). Chlorophenol red and FeCl_3 were omitted. *Thiobacillus perometabolis* THI 023 was grown in medium 528 (Gherna & Pienta, 1980) with 1% (w/v) $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ and 0.5% (w/v) yeast extract (Difco) and 0.5 ml per 100 ml of trace metal solution at pH 6.8. Bromothymol blue, FeCl_3 and heavy metal solution were omitted. *Thiobacillus delicatus* THI 091 and *Thiobacillus intermedius* THI 101 were grown in YES medium (Mizoguchi *et al.*, 1976) and in a basal salt medium (London, 1963) supplemented with 0.5% (w/v) $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ and 0.1% (w/v) yeast extract (Difco), respectively.

Thiosulphate (1%, w/v) was used as growth substrate for *T. thioparus* THI 111 and THI 112 in SM basal salt medium, and for *Thiobacillus denitrificans* THI 151 and THI 152 and *Thiobacillus neapolitanus* THI 121 and THI 122 in medium 290 (Gherna & Pienta, 1980). SM basal salt medium contained (%, w/v): KH_2PO_4 , 0.15; Na_2HPO_4 , 0.45; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01; NH_4Cl , 0.03; 0.5 ml per 100 ml of the trace metal solution; pH 7.5. *Thiobacillus denitrificans* THI 151 and THI 152 were cultured anaerobically in a 5 l Erlenmeyer flask which was filled with liquid medium 290 to which 0.5% (w/v) KNO_3 and 0.05% (w/v) NaHCO_3 were added. *Thiobacillus ferrooxidans* THI 132 was grown in a 10 l carboy containing 6 l of ferrous salt medium 9K (Silverman & Lundgren, 1959) under forced aeration. This strain could not grow in mineral salt/glucose medium. *Thiobacillus thiooxidans* THI 141 and THI 142 and *Thiobacillus concretivorus* THI 143 were grown in elemental sulphur salt medium 6 (Waksman, 1922). Unless otherwise mentioned, organisms were cultured in a 5 l shake flask containing 1 l of liquid medium on a rotary shaker. Organisms were grown at 30 °C except for *T. organoparus* THI 071 which was grown at 25 °C.

Solutions of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, NaHCO_3 and glucose were autoclaved separately from other components of the media. Powdered sulphur was steamed for 1 h on three successive days.

Extraction and analysis of ubiquinones. Cells were collected by centrifugation, washed with distilled water and freeze-dried. Quinones were analysed in the dried cells (0.2 to 3 g) according to Katayama-Fujimura & Kuraishi (1980). Extraction with ethanol/ether was repeated when sufficient quinone was not recovered. After purification of the quinone by TLC developed with benzene, it was analysed by Shimadzu-LKB 9000 mass spectrometry using a direct insertion probe with an electron energy of 70 eV and a temperature range of 160 to 190 °C.

Extraction and analysis of cellular fatty acid. Cells were collected by centrifugation in the late-exponential phase for facultative chemolithotrophs and chemoorganotrophs, washed with distilled water and freeze-dried. Obligate chemolithotrophs were harvested in the stationary phase. Cellular fatty acids were extracted from alkaline saponified materials, esterified with diazomethane, and analysed by GLC as previously described (Katayama & Kuraishi, 1978).

Bromination (James & Martin, 1956) and hydrogenation were used for the identification of unsaturated fatty acids. Refluxing with HCl /methanol was used to identify cyclopropane acids (Uchida & Mogi, 1972). Gas chromatography-mass spectrometry was also used to identify the cellular fatty acids.

DNA base composition. DNA was isolated and purified (Marmur, 1961) from cells grown in the appropriate medium for each strain and harvested in the late-exponential phase. DNA base composition was calculated from

Table 1. *Bacterial strains studied*

Species	Laboratory no.	Source†
<i>Thiobacillus perometabolis</i>	THI 022	ATCC 27793
<i>Thiobacillus perometabolis</i>	THI 023*	IAM 12132 (ATCC 23370)
<i>Thiobacillus</i> sp. A2	THI 041	ATCC 25364
<i>Thiobacillus organoparus</i>	THI 071	ATCC 27977
<i>Thiobacillus acidophilus</i>	THI 061	ATCC 27807
<i>Thiobacillus delicatus</i>	THI 091	T. Mizoguchi, Chemical Research Institute of Non-Aqueous Solution, Tohoku University, Sendai, Japan (IAM 12624)
<i>Thiobacillus intermedius</i>	THI 101*	IAM 12131 (ATCC 15466)
<i>Thiobacillus denitrificans</i>	THI 151	ATCC 23642
<i>Thiobacillus denitrificans</i>	THI 152	ATCC 23644
<i>Thiobacillus thioparus</i>	THI 111*	IAM 12113 (ATCC 8158)
<i>Thiobacillus thioparus</i>	THI 112	Laboratory strain TK 21 (Katayama & Kuraishi, 1978)
<i>Thiobacillus neapolitanus</i>	THI 121	IAM 12116 (ATCC 23640)
<i>Thiobacillus neapolitanus</i>	THI 122	IAM 12117 (ATCC 23641)
<i>Thiobacillus ferrooxidans</i>	THI 132	IAM 12130 (ATCC 19859)
<i>Thiobacillus thiooxidans</i>	THI 141	IAM 12133 (ATCC 8085)
<i>Thiobacillus thiooxidans</i>	THI 142	IAM 12134 (ATCC 19377)
<i>Thiobacillus concretivorus</i>	THI 143	M. Yagisawa, Fermentation Research Institute, Agency of Industrial Science and Technology, Ibaraki, Japan (ATCC 15494)
<i>Thiobacillus rubellus</i>	THI 001	T. Mizoguchi; (IAM 12623)
<i>Thiobacillus trautweinii</i>	THI 081	IAM 12135 (NCIB 9549, LMD 38.20)
<i>Thiobacillus trautweinii</i>	THI 082	IAM 12564 (NCIB 9550, LMD 38.21)

* Type strain.

† ATCC, American Type Culture Collection, Rockville, U.S.A.; IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; NCIB, National Collection of Industrial Bacteria, Aberdeen; LMD, Delft University of Technology, Laboratory of Microbiology, Delft, The Netherlands.

the thermal denaturation temperature (Marmur & Doty, 1962) determined with a Gilford model 250 spectrophotometer. DNA from *Micrococcus luteus* IAM 1056 (ATCC 4698), which contains 71.9 mol% G + C (Lee *et al.*, 1956), was used as the reference.

Protein. Cellular protein was determined by the Lowry method after precipitation at 90 °C for 15 min with 5% (w/v) trichloroacetic acid.

Chemicals. Fatty acids for reference standards were purchased from Nakarai Chemical Ltd, Kyoto, Japan; Tokyo Kasei, Tokyo, Japan; Applied Science Laboratories, State College, Penna, U.S.A. and Sigma. Oleic acid and *cis*-vaccenic acid were obtained from Kokusan Kagaku Co., Tokyo, Japan and Sigma, respectively. All the chemicals used were commercial products.

RESULTS

Ubiquinones

TLC analyses of ethanol/ether extracts from the test strains revealed ubiquinones as the sole respiratory quinones. The mass spectra of all the quinone samples showed intense peaks at *m/e* 235 and 197 derived from the pyriliium and benzeliium (Muraca *et al.*, 1967). The mass spectra in the high mass region contained strong peaks corresponding to the molecular ion (M^+). The presence of dihydrogenated ubiquinones was excluded in several samples by examining with high performance liquid chromatography fitted with a Zorbax ODS (Shimadzu-Du Pont Co., Kyoto, Japan) pre-packed column (4.6 × 250 mm) and methanol/isopropyl ether (85:15, v/v) as the mobile phase at 1.0 ml min⁻¹ and monitored at 275 nm.

From the mass spectral analyses of the ubiquinones (Table 2), the thiobacilli were divided into two major groups. Several facultative chemolithotrophs, i.e. *T. perometabolis* THI 022, *Thiobacillus* sp. A2 THI 041, *T. novellus* THI 031, *T. organoparus* THI 071 and *T. acidophilus*

Table 2. Occurrence of ubiquinones in the genus *Thiobacillus*

Species	Strain	Ubiquinone*		
		Q-8	Q-9	Q-10
<i>T. perometabolis</i>	THI 022	—	—	+++
<i>Thiobacillus</i> sp. A2	THI 041	—	—	+++
<i>T. novellus</i>	THI 031	—	—	+++†
<i>T. organoparus</i>	THI 071	—	—	+++
<i>T. acidophilus</i>	THI 061	—	—	+++
<i>T. delicatus</i>	THI 091	+++	—	—
<i>T. perometabolis</i>	THI 023	+++	—	—
<i>T. intermedius</i>	THI 101	+++	—	—
<i>T. denitrificans</i>	THI 151	+++	—	—
<i>T. denitrificans</i>	THI 152	+++	—	—
<i>T. thioparus</i>	THI 111	+++	—	—
<i>T. thioparus</i>	THI 112	+++	—	—
<i>T. neapolitanus</i>	THI 121	+++	—	—
<i>T. neapolitanus</i>	THI 122	+++	—	—
<i>T. ferrooxidans</i>	THI 132	+++	+	—
<i>T. thiooxidans</i>	THI 141	+++	—	—
<i>T. thiooxidans</i>	THI 142	+++	—	—
<i>T. concretivorus</i>	THI 143	+++	—	—

* The main component of ubiquinone of each strain is denoted by +++. A significant peak by mass spectrum is denoted by +.

† Katayama-Fujimura & Kuraishi (1980).

Table 3. DNA base composition of species of the genus *Thiobacillus*, *T. trautweinii* and *T. rubellus*

Species	Strain	G + C (mol %)	G + C* (mol %)	References
Q-10				
<i>T. perometabolis</i>	THI 022	67.8	65.0	Myers & Millar (1975)
<i>Thiobacillus</i> sp. A2	THI 041	67.0	65–68	Taylor & Hoare (1969)
<i>T. novellus</i>	THI 031		68	Jackson <i>et al.</i> (1968); Taylor & Hoare (1969)
			67.3	Katayama-Fujimura & Kuraishi (1980)
<i>T. organoparus</i>	THI 071	62.6		
<i>T. acidophilus</i>	THI 061	ND	62.9–63.2	Guay & Silver (1974)
Q-8				
<i>T. delicatus</i>	THI 091	66.0	67 ± 3	Mizoguchi <i>et al.</i> (1976)
<i>T. perometabolis</i>	THI 023	65.0		
<i>T. intermedius</i>	THI 101	64.9		
<i>T. denitrificans</i>		ND	64	
<i>T. thioparus</i>	THI 111	ND	62.0	Jackson <i>et al.</i> (1968)
<i>T. thioparus</i>	THI 112		61.0	Katayama & Kuraishi (1978)
<i>T. neapolitanus</i>		ND	56	
<i>T. ferrooxidans</i>		ND	57	
<i>T. thiooxidans</i>		ND	52	
<i>T. concretivorus</i>		ND	51–52	Jackson <i>et al.</i> (1968)
Q-9				
<i>T. trautweinii</i>	THI 081	ND	66	
Q-8				
<i>T. trautweinii</i>	THI 082	ND	66.4	Yamasato & Akagawa (unpublished)
Q-10, Q-9				
<i>T. rubellus</i>	THI 001	68.8	65 ± 3	Mizoguchi <i>et al.</i> (1976)

* Cited from literature.

ND, Not determined.

Fig. 1. Cellular fatty acid composition of *T. acidophilus* THI 061 calculated on the basis of total non-hydroxylated fatty acids and hydroxy fatty acids relative to the total of non-hydroxylated acid (a), and those calculated on the basis of the total of cellular fatty acids (b) expressed as a percentage. In fatty acid designations, the first number indicates the number of carbon atoms and the second the number of double bonds; cyc indicates cyclopropane fatty acid; 3-OH indicates β -hydroxy fatty acid. The 18:1 acid is *cis*-vaccenic acid.

THI 061, contained Q-10. Q-8 was a component of the remaining facultative chemolithotrophic strains, i.e. *T. delicatus* THI 091, *T. perometabolis* THI 023 and *T. intermedius* THI 101, and all obligate chemolithotrophic strains, i.e. *T. denitrificans* THI 151 and THI 152, *T. thioparus* THI 111 and THI 112, *T. neapolitanus* THI 121 and THI 122, *T. ferrooxidans* THI 132, *T. thiooxidans* THI 141 and THI 142 and *T. concretivorus* THI 143.

DNA base composition

The DNA base composition was determined for *T. perometabolis* THI 022 and THI 023, *Thiobacillus* sp. A2 THI 041, *T. organoparus* THI 071, *T. delicatus* THI 091, *T. intermedius* THI 101 and *T. rubellus* THI 001. These results are given in Table 3, together with some published data. DNA base compositions of strains of *Thiobacillus* species were in the range of 51 to 68 mol% G + C. Those species with Q-10 contained 63 to 68 mol% G + C, whereas those species with Q-8 varied from 51 to 66 mol% G + C.

Fatty acid composition

The relative retention time (r.r.t.) of the monounsaturated straight chain fatty acid with C₁₈ (18:1) of thiobacilli (r.r.t. = 2.217 as average value) corresponded to that of a standard sample of *cis*-vaccenic (*cis*-11, 12-octadecenoic) acid (r.r.t. = 2.200) upon GLC using a 5 m long column packed with 10% diethylene glycol succinate. The r.r.t. of a standard sample of oleic acid was 2.155 under the same analytical conditions. Cyclopropane acid with C₁₉ (19_{cyc}) was biosynthesized from *cis*-vaccenic acid (O'Leary, 1959; Hofmann & Liu, 1960). The relative amounts of cyclopropane acids and monounsaturated acids were known to change during batch culture growth (Law *et al.*, 1963), therefore, monounsaturated straight chain acids and the corresponding cyclopropane acids were presented as the sum of their values in addition to their individual values.

The proportion of the hydroxy fatty acids relative to the total cellular fatty acids varied markedly with the strain. In Gram-negative bacteria, 2- and 3-hydroxy fatty acids have been

Table 4. *Cellular fatty acid composition of the facultatively chemolithotrophic thiobacilli with Q-10 (group I)*

Components of cellular fatty acids* were calculated on the basis of non-hydroxylated acids. Hydroxy fatty acids are presented relative to the total of non-hydroxylated acids.

Species	Strain	Non-hydroxylated acid												Hydroxy acid		
		12:0	14:0	15:0	16:0	17:0	18:0	12:1	16:1	18:1	17 _{cyc}	19 _{cyc}	16:1 + 17 _{cyc}	18:1 + 19 _{cyc}	3-OH 10:0	3-OH 14:0
Group I-1																
<i>T. perometabolis</i>	THI 022	tr	—	—	7	—	2	4	tr	86	—	—	tr	86	9	—
<i>Thiobacillus</i> sp. A2	THI 041	—	tr	tr	6	tr	4	13	tr	73	—	2	tr	75	34	—
<i>T. novellus</i>	THI 031†	—	tr	tr	9	2	2	—	tr	71	—	14	tr	85	—	—
Group I-2																
<i>T. organoparus</i>	THI 071	tr	tr	tr	7	—	4	—	2	42	—	43	2	85	—	20
<i>T. acidophilus</i>	THI 061	tr	6	1	12	—	8	—	3	8	2	60	5	68	—	56

tr, Indicates trace amounts (less than 1%).

* In fatty acid designations, the first number indicates the number of carbon atoms and the second the number of double bonds; cyc, indicates cyclopropane fatty acid; 3-OH, indicates β -hydroxy fatty acid. The 18:1 acid is *cis*-vaccenic acid.

† Cited from Katayama-Fujimura & Kuraishi (1980).

Table 5. *Cellular fatty acid composition of the facultatively chemolithotrophic thiobacilli with Q-8 (group II)*

Components of cellular fatty acids* were calculated on the basis of non-hydroxylated acids. Hydroxy fatty acids are presented relative to the total of non-hydroxylated acids.

Species	Strain	Non-hydroxylated acid												Hydroxy acid			
		12:0	14:0	15:0	16:0	17:0	18:0	16:1	18:1	17 _{cyc}	19 _{cyc}	i16:0	16:1 + 17 _{cyc}	18:1 + 19 _{cyc}	3-OH 10:0	3-OH 12:0	3-OH i10:0
Group II																	
<i>T. delicatus</i>	THI 091	tr	tr	tr	33	—	tr	6	24	25	9	2	31	33	13	—	1
<i>T. perometabolis</i>	THI 023	tr	tr	tr	37	tr	1	16	37	5	2	tr	21	39	19	9	tr
<i>T. intermedius</i>	THI 101	—	tr	—	41	—	tr	16	35	5	2	—	21	36	5	5	—

tr, Indicates trace amount (less than 1%).

* The prefix *i* indicates *iso* branching; other designations are as indicated in Table 4.

found as components of lipopolysaccharides and amino lipids (Hancock *et al.*, 1970; Key *et al.*, 1970; Koeltzow & Conrad, 1971; Knoche & Shively, 1972). The total hydroxy fatty acids were therefore calculated relative to the total non-hydroxylated acids after the samples were separated into 2- and 3-hydroxy and non-hydroxylated fatty acids by TLC and developed with hexane/ethylether (80:20, v/v). Figure 1 shows a comparison between cellular fatty acids calculated on the basis of the total of non-hydroxylated fatty acids and hydroxy fatty acid relative to the total of non-hydroxylated acid (*a*) and those calculated on the basis of the total of cellular fatty acid (*b*).

Table 6. Cellular fatty acid composition of the obligate chemolithotrophic thiobacilli with Q-8 (group III)

Components of cellular fatty acids* were calculated on the basis of non-hydroxylated acids. Hydroxy fatty acids are presented relative to the total of non-hydroxylated acids.

Species	Strain	Non-hydroxylated acid														Hydroxy acid					
		10:0	12:0	13:0	14:0	15:0	16:0	17:0	18:0	12:1	14:1	15:1	16:1	18:1	17 ^{cyc}	19 ^{cyc}	16:1 + 17 ^{cyc}	18:1 + 19 ^{cyc}	3-OH 10:0	3-OH 12:0	3-OH 14:0
Group III-1																					
<i>T. denitrificans</i>	THI 151	2	4	—	tr	tr	39	—	tr	—	—	—	31	2	19	—	50	2	5	1	—
<i>T. denitrificans</i>	THI 152	tr	4	—	tr	1	44	—	tr	—	—	—	29	3	17	—	46	3	5	2	—
<i>T. thioparus</i>	THI 111†	3	6	—	tr	1	41	1	2	—	—	—	8	3	34	—	42	3	6	5	—
<i>T. thioparus</i>	THI 112†	4	6	—	tr	1	38	1	2	—	—	—	12	3	33	—	45	3	5	5	—
Group III-2																					
<i>T. neapolitanus</i>	THI 121	—	4	tr	8	2	35	tr	1	tr	3	tr	13	16	8	9	21	25	tr	17	—
<i>T. neapolitanus</i>	THI 122	—	4	tr	10	2	30	1	1	tr	1	—	23	21	4	2	28	23	tr	10	—
Group III-3																					
<i>T. ferrooxidans</i>	THI 132	—	4	—	1	tr	17	2	7	—	—	—	14	44	2	9	16	53	—	—	15
<i>T. thiooxidans</i>	THI 141	—	6	—	tr	tr	16	3	5	—	—	—	4	6	18	41	23	47	—	—	11
<i>T. thiooxidans</i>	THI 142	—	5	tr	tr	tr	9	4	4	—	—	—	5	12	12	48	17	59	—	—	9
<i>T. concretivorans</i>	THI 143	—	10	tr	tr	tr	14	3	5	—	—	—	8	9	12	39	20	48	—	—	20

tr, Indicates trace amount (less than 1 %).

* Fatty acid designations are as indicated in Table 4.

† Cited from Katayama & Kuraishi (1978).

Facultatively chemolithotrophic thiobacilli with Q-10 characteristically possessed a high proportion of [18:1 + 19_{cyc}] which composed 70 to 85% of the non-hydroxylated acid, and 3-hydroxydecanoic acid (3-OH 10:0) or 3-hydroxytetradecanoic acid (3-OH 14:0) (Table 4). However, in *T. novellus* THI 031, hydroxy fatty acids were not detected. The species of facultative chemolithotrophs with Q-8 (Table 5), and all species of obligate chemolithotrophs (Table 6) had hexadecanoic acid (16:0), [hexadecenoic acid (16:1) + cyclopropane acid of C₁₇ (17_{cyc})] with or without [18:1 + 19_{cyc}] as major non-hydroxylated acids. Hydroxy fatty acids [3-OH 10:0, 3-hydroxydodecanoic acid (3-OH 12:0) or 3-OH 14:0] showed a characteristic distribution in this taxon.

Grouping of the species in the genus Thiobacillus

Thiobacillus species were divided into two major groups based on the ubiquinone systems. Facultatively chemolithotrophic thiobacilli with Q-10 constituted group I. *Thiobacillus* species with Q-8 included several species of facultatively chemolithotrophic and obligate chemolithotrophic thiobacilli. Therefore, these organisms could be divided nutritionally into facultatively chemolithotrophic species with Q-8 (group II) and obligate chemolithotrophic thiobacilli (group III).

Group I. Facultative chemolithotrophs with Q-10

This group included *T. perometabolis* THI 022, *Thiobacillus* sp. A2 THI 041, *T. novellus* THI 031, *T. organoparus* THI 071 and *T. acidophilus* THI 061 (Table 4). Although *T. perometabolis* THI 022 was described as a chemoorganotrophic bacterium by the original isolators (Myers & Millar, 1975), this organism grew autotrophically in mineral salt/thiosulphate (0.5%, w/v) medium. The final cell yield was 32 mg l⁻¹ as protein, which almost corresponded to the yield (57 mg l⁻¹) determined for *T. novellus* THI 031 under the same cultural system (Katayama-Fujimura & Kuraishi, 1980). The major component of non-hydroxylated fatty acid was [18:1 + 19_{cyc}] (Table 4). DNA base compositions of *T. perometabolis* THI 022, *Thiobacillus* sp. A2 THI 041 and *T. novellus* THI 031 were 67.8, 67.0 and 67.3 mol% G + C, respectively (group I-1). *Thiobacillus perometabolis* THI 022 and *Thiobacillus* sp. A2 THI 041 had 3-OH 10:0 as a hydroxy fatty acid, and dodecenoic acid (12:1) as a minor component. On the other hand, *T. organoparus* THI 071 and *T. acidophilus* THI 061, which are acidophilic species, contained 62.6 and 62.9 to 63.2 mol% G + C, respectively (Guay & Silver, 1974, for *T. acidophilus*) (group I-2). Both organisms contained 3-OH 14:0 as major cellular fatty acid component.

Group II. Facultative chemolithotrophs with Q-8

This group included *T. delicatus* THI 091, *T. perometabolis* THI 023 and *T. intermedius* THI 101 (Table 5). *Thiobacillus perometabolis* THI 023 was originally described as a chemoorganotrophic bacterium (London & Rittenberg, 1967), but it grew autotrophically in a medium supplemented with thiosulphate and the maximal cell yield was 40 mg l⁻¹ as protein. DNA base compositions of these organisms were quite similar (Table 3). Major components of the cellular fatty acid composition were 16:0, [16:1 + 17_{cyc}] and [18:1 + 19_{cyc}]. These organisms had 3-OH 10:0 as hydroxy fatty acid. *Thiobacillus perometabolis* THI 023 and *T. intermedius* THI 101 also contained 3-OH 12:0. The gas chromatograms of the 3-hydroxy fatty acids fraction separated by TLC from *T. perometabolis* THI 023 and *T. delicatus* THI 091 showed a minor component, which emerged ahead of 3-OH 10:0 on polar and non-polar columns. The component was identified as 3-hydroxy-8-methylnonanoic acid (3-OH i10:0) by mass spectrometry and showed a faint peak of molecular ion (*m/e* 202) and a rather weak but significant peak at *m/e* 137 (M⁺-65) (Moss *et al.*, 1973) in addition to the characteristic peaks of 3-hydroxy fatty acids [*m/e* 103, *m/e* 152 (M⁺/50)].

Table 7. Ubiquinone system and cellular fatty acid composition of *T. trautweinii* and *T. rubellus*

Components of cellular fatty acids* were calculated on the basis of non-hydroxylated acids. Hydroxy fatty acids are presented relative to the total of non-hydroxylated acids.

Species	Strain	Ubiquinone†			Non-hydroxylated acid										Hydroxy acid								
		Q-8	Q-9	Q-10	10:0	12:0	14:0	15:0	16:0	17:0	18:0	16:1	18:1	17 ^{cyc}	19 ^{cyc}	16:1 + 17 ^{cyc}	18:1 + 19 ^{cyc}	3-OH 10:0	3-OH 12:0	3-OH 14:0	2-OH 12:0	unknown (3-OH)	
<i>T. trautweinii</i>	THI 081	—	+++	—	tr	13	1	tr	21	tr	1	24	37	tr	—	25	37	25	2	—	—	—	—
<i>T. trautweinii</i>	THI 082	+++	—	—	—	16	10	tr	24	2	3	37	6	3	—	40	6	6	7	59	21	—	—
<i>T. rubellus</i>	THI 001	—	+	+++	—	—	tr	1	5	—	10	tr	83	—	—	tr	83	—	—	—	—	—	3

tr, Indicates trace amount (less than 1 %).

* The prefix 2-OH indicates α -hydroxy group; other designations are as indicated in Tables 4 and 5.

† The main component of ubiquinone of each strain is denoted by +++. A significant peak by mass spectrum is denoted by +.

Group III. Obligate chemolithotrophs with Q-8

The major non-hydroxylated fatty acids were 16:0 [16:1 + 17_{cyc}] with or without [18:1 + 19_{cyc}] (Table 6). This group was further divided into three subgroups based on the proportion of major non-hydroxylated fatty acids together with the constituents of 3-hydroxy fatty acid. Group III-1 consisted of *T. denitrificans* THI 151 and THI 152 and *T. thioparus* THI 111 and THI 112, which contained 16:0 and [16:1 + 17_{cyc}] as major components, and 3-OH 10:0 and 3-OH 12:0 as hydroxy fatty acids. DNA base compositions of these organisms ranged from 61 to 64 mol% G + C (Jackson *et al.*, 1968; Katayama & Kuraishi, 1978).

Thiobacillus neapolitanus THI 121 and THI 122 formed another subgroup (Group III-2). These organisms had 16:0 [16:1 + 17_{cyc}] and [18:1 + 19_{cyc}] as a major non-hydroxylated fatty acid, and 3-OH 12:0 as hydroxy acid. Monounsaturated straight chain acids with C₁₂, C₁₄ and C₁₅, were observed in these organisms as minor components. DNA base composition was 56 mol% G + C (Jackson *et al.*, 1968).

Group III-3 included *T. ferrooxidans* THI 132, *T. thiooxidans* THI 141 and THI 142 and *T. concretivorus* THI 143. The major non-hydroxylated fatty acids were [18:1 + 19_{cyc}], which was the most significant component among the three subgroups [16:1 + 17_{cyc}] and 16:0. The organisms had 3-OH 14:0 as hydroxy acid. DNA base composition of *T. thiooxidans* and *T. concretivorus* ranged from 51 to 52 mol% G + C, and that of *T. ferrooxidans* was 57 mol% G + C (Jackson *et al.*, 1968).

Chemotaxonomic characteristics of T. trautweinii and T. rubellus

Thiobacillus trautweinii THI 081 contained Q-9 and THI 082 contained Q-8 (Table 7). A major component of the cellular fatty acids of *T. trautweinii* THI 081 was [18:1 + 19_{cyc}] followed by [16:1 + 17_{cyc}] and 16:0, with 3-OH 10:0 and 3-OH 12:0 as hydroxy fatty acids (Table 7). On the other hand, cellular fatty acid composition of *T. trautweinii* THI 082 was [16:1 + 17_{cyc}], followed by [18:1 + 19_{cyc}] and 16:0, with 3-OH 10:0, 3-OH 12:0 and 3-OH 14:0 and 2-OH 12:0 as hydroxy fatty acids. These strains were chemoorganotrophs, and their growth was not supported in a mineral salt medium supplemented with thiosulphate as described by Starkey (1935).

Thiobacillus rubellus THI 001 contained Q-10 as the major isoprenologue with a trace amount of Q-9. The major component of cellular fatty acid of this organism was [18:1 + 19_{cyc}]. Mizoguchi *et al.* (1976) described *T. rubellus* as a facultative chemolithotroph, but no autotrophic growth was observed in our experiments.

DISCUSSION

Chemotaxonomic characteristics provide valuable information for the differentiation and classification of bacterial groups (Ikemoto *et al.*, 1978; Yamada *et al.*, 1969, 1976*a, b*; Collins *et al.*, 1977, 1979, 1980). The presence of Q-8 has been reported in *T. thiooxidans*, *T. thioparus* (Cook & Umbreit, 1963) and *T. ferrooxidans* (Short *et al.*, 1969), whereas Q-10 was found in *T. novellus* (Katayama-Fujimura & Kuraishi, 1980).

A summary of the classification of the genus *Thiobacillus* on the basis of chemotaxonomic characteristics is shown in Fig. 2. The ubiquinone system seems to be useful for preliminary identification of thiobacilli, especially for facultative chemolithotrophs. Hydroxy fatty acids showed characteristic distribution within the species of each group. Therefore, further identification could be based on both hydroxy fatty acids and physiological characteristics.

Agate & Vishniac (1973) described cellular fatty acid compositions of thiobacilli, which, together with DNA base compositions (Jackson *et al.*, 1968) were used for the groupings in *Bergey's Manual of Determinative Bacteriology*, 8th edition (Vishniac, 1974). Agate & Vishniac (1973) reported the cellular fatty acid compositions as follows: in *T. neapolitanus* and *T. ferrooxidans*, 14:1, 16:1, 14:0 and 16:0, in *T. denitrificans* and *T. intermedius*,

Chemolithotroph	Ubiquinone	Hydroxy fatty acid	Species	Remarks	Group
Facultative	Q-10	None	<i>T. novellus</i>	Atypical strain	I-1
		3-OH 10:0	<i>Thiobacillus</i> A2		
		3-OH 14:0	<i>T. perometabolis</i>		Acidophilic
	Q-8	3-OH 10:0	<i>T. organoparus</i>		
		3-OH 10:0, 3-OH 12:0	<i>T. acidophilus</i>	Type strain	II
		3-OH 10:0, 3-OH 12:0	<i>T. delicatus</i>		
Obligate	Q-8	3-OH 10:0, 3-OH 12:0	<i>T. perometabolis</i>		III-1
		3-OH 12:0	<i>T. intermedius</i>		
		3-OH 12:0	<i>T. denitrificans</i>	Acidophilic	III-2
	3-OH 14:0	<i>T. thioparus</i>			
		<i>T. neapolitanus</i>			
			<i>T. ferrooxidans</i>		III-3
		<i>T. thiooxidans</i>			
			<i>T. concretivorus</i>		

Fig. 2. Proposed key for the identification of the species of the genus *Thiobacillus*.

15:0, 17:0 and 17:1, in *T. thiooxidans*, 14:0, 14:1, 12:0 and 11:1. However, we did not find the same patterns of cellular fatty acids in our present and previous studies (Katayama & Kuraishi, 1978; Katayama-Fujimura & Kuraishi, 1980). Levin (1971, 1972) described the cellular fatty acid composition of *T. novellus* and *T. thiooxidans*. These results were similar to ours for non-hydroxylated fatty acids, but Levin did not detect hydroxy fatty acids in *T. thiooxidans*.

The composition of cellular fatty acids was more readily apparent when expressed relative to the total non-hydroxylated acid [method (a) above] than when calculated on the basis of total fatty acids. We consider the presentation is valuable when the proportion of hydroxy fatty acid to non-hydroxylated fatty acids varies considerably with the strains, and also when cellular fatty acids are compared between a large number of strains.

Thiobacillus sp. A2 was isolated by Taylor & Hoare (1969) as a strain of *T. novellus*. Differences between *Thiobacillus* sp. A2 and *T. novellus* were found in the growth rate and utilization of organic compounds. The presence of hydroxy fatty acids further revealed a difference between these organisms; *Thiobacillus* sp. A2 had 3-OH 10:0, while in *T. novellus* (Levin, 1972; Katayama-Fujimura & Kuraishi, 1980) hydroxy fatty acid was not detected. *Thiobacillus* sp. A2 TH1 041 was similar to *T. perometabolis* TH1 022 in cellular fatty acid composition, especially in the presence of 3-OH 10:0 and 12:1 which is a significant component. Further research on the physiological characteristics of these bacteria is needed for the emendation of *Thiobacillus* sp. A2 TH1 041 and *T. perometabolis* TH1 022.

The presence of 3-OH 14:0 in acidophilic strains in groups I-2 and III-3 was interesting, even though they differed from each other in the ubiquinone system. Knoche & Shively (1972) showed the presence of *cis*-11,12-methylene-2-hydroxyoctadecanoic acid in *T. thiooxidans*, which linked ornithine-3-hydroxy hexadecanoic acid at hydroxy group of 3-hydroxy acid. We could not detect 3-hydroxyhexadecanoic acid by GLC after separation by TLC nor 2-hydroxy acid in our experiments.

In *Bergey's Manual of Determinative Bacteriology*, 8th edition (Vishniac, 1974), *T. trautweinii* is excluded from the genus *Thiobacillus* because it does not obtain energy by the oxidation of thiosulphate into tetrathionate. The exclusion of *T. trautweinii* from the genus was deemed reasonable on the basis of the quinone system and cellular fatty acid composition, especially the presence of the hydroxy fatty acid, as well as physiological characteristics (Starkey, 1935; Hutchinson *et al.*, 1965, 1969). *Thiobacillus trautweinii* TH1 081 and TH1 082 were recognized as a strain of *Pseudomonas* and a strain of *Alcaligenes*, respectively, in the catalogue of strains of the National Collection of Industrial Bacteria (Bousfield & Graham, 1975).

Thiobacillus rubellus is a chemoorganotroph. The organism forms a characteristic pink to red colony and has an ability to grow in mineral salt/methanol medium. Therefore, *T. rubellus* TH1 001 should be transferred to another bacterial group, such as the methanol-assimilating bacteria described by Urakami & Komagata (1979).

We thank Dr T. Suto, Nippon Veterinary and Zootechnical College, and Dr K. Komagata, The Institute of Applied Microbiology, University of Tokyo, for their helpful discussion and encouragement, and Dr Y. Yamada, Shizuoka University, for his technical advice on the determination of quinones. We also thank Dr K. Uchida, The Institute of Applied Microbiology, University of Tokyo, for his helpful advice on fatty acids and quinones. We further thank Ms P. Pienta, Asst Curator of Bacteriology, American Type Culture Collection, for her beneficial advice on *T. perometabolis*. We are indebted to Dr T. Mizoguchi and Dr M. Yagisawa, for their gift of cultures. The technical assistance of S. Nakayama and S. Segawa in carrying out certain experiments is acknowledged.

REFERENCES

- AGATE, A. D. & VISHNIAC, W. (1973). Characterization of *Thiobacillus* species by gas-liquid chromatography of cellular fatty acids. *Archiv für Mikrobiologie* **89**, 257-267.
- BOUSFIELD, I. J. & GRAHAM, S. D. (1975). In *The National Collection of Industrial Bacteria, Catalogue of Strains*, 3rd edn, p. 28 and p. 184. London: HMSO.
- COLLINS, M. D., PIROUZ, T., GOODFELLOW, M. & MINNIKIN, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *Journal of General Microbiology* **100**, 221-230.
- COLLINS, M. D., GOODFELLOW, M. & MINNIKIN, D. E. (1979). Isoprenoid quinones in the classification of coryneform and related bacteria. *Journal of General Microbiology* **110**, 127-136.
- COLLINS, M. D., GOODFELLOW, M. & MINNIKIN, D. E. (1980). Fatty acid, isoprenoid quinone and polar lipid composition in the classification of *Curtobacterium* and related taxa. *Journal of General Microbiology* **118**, 29-37.
- COOK, T. M. & UMBREIT, W. W. (1963). The occurrence of cytochrome and coenzyme Q in *Thiobacillus thiooxidans*. *Biochemistry* **2**, 194-196.
- GHERNA, R. L. & PIENTA, P. (1980). Media. In *The American Type Culture Collection. Catalogue of Strains I*, 14th edn, pp. 511-559. Edited by H. D. Hatt, M. J. Gant & J. Piper. Rockville: American Type Culture Collection.
- GUAY, R. & SILVER, M. (1974). *Thiobacillus acidophilus* sp. nov.; isolation and some physiological characteristics. *Canadian Journal of Microbiology* **21**, 281-288.
- HANCOCK, I. C., HUMPHREYS, G. O. & MEADOW, P. M. (1970). Characterisation of the hydroxy acids of *Pseudomonas aeruginosa* 8602. *Biochimica et biophysica acta* **202**, 389-391.
- HOFMANN, K. & LIU, T. Y. (1960). Lactobacillic acid biosynthesis. *Biochimica et biophysica acta* **37**, 364-365.
- HUTCHINSON, M., JOHNSTONE, K. I. & WHITE, D. (1965). The taxonomy of certain thiobacilli. *Journal of General Microbiology* **41**, 357-366.
- HUTCHINSON, M., JOHNSTONE, K. I. & WHITE, D. (1966). Taxonomy of the acidophilic thiobacilli. *Journal of General Microbiology* **44**, 373-381.
- HUTCHINSON, M., JOHNSTONE, K. I. & WHITE, D. (1967). Taxonomy of anaerobic thiobacilli. *Journal of General Microbiology* **47**, 17-23.
- HUTCHINSON, M., JOHNSTONE, K. I. & WHITE, D. (1969). Taxonomy of the genus *Thiobacillus*: the outcome of numerical taxonomy applied to the group as a whole. *Journal of General Microbiology* **57**, 397-410.
- IKEMOTO, S., KURAISHI, H., KOMAGATA, K., AZUMA, R., SUTO, T. & MUROOKA, H. (1978). Cellular fatty acid composition in *Pseudomonas* species. *Journal of General and Applied Microbiology* **24**, 199-213.
- JACKSON, J. F., MORIARTY, D. J. W. & NICHOLAS, D. J. D. (1968). Deoxyribonucleic acid base composition and taxonomy of thiobacilli and some nitrifying bacteria. *Journal of General Microbiology* **53**, 53-60.
- JAMES, A. T. & MARTIN, A. J. P. (1956). Gas-liquid chromatography: the separation and identification of the methyl esters of saturated and unsaturated acids from formic acid to *n*-octadecanoic acid. *Biochemical Journal* **63**, 144-152.
- KATAYAMA, Y. & KURAISHI, H. (1978). Characteristics of *Thiobacillus thioarvus* and its thiocyanate assimilation. *Canadian Journal of Microbiology* **24**, 804-810.
- KATAYAMA-FUJIMURA, Y. & KURAISHI, H. (1980). Characterization of *Thiobacillus novellus* and its thiosulfate oxidation. *Journal of General and Applied Microbiology* **26**, 357-367.
- KEY, B. A., GRAY, G. W. & WILKINSON, S. G. (1970). The purification and chemical composition of the lipopolysaccharide of *Pseudomonas alcaligenes*. *Biochemical Journal* **120**, 559-566.
- KNOCH, H. W. & SHIVELY, J. M. (1972). The structure of an ornithine-containing lipid from *Thiobacillus thiooxidans*. *Journal of Biological Chemistry* **247**, 170-178.
- KOELTZOW, D. E. & CONRAD, H. E. (1971). Structural heterogeneity in the lipopolysaccharide of *Aerobacter aerogenes* NCTC 243. *Biochemistry* **10**, 214-224.
- LAW, J. H., ZALKIN, H. & KANESHIRO, T. (1963). Transmethylation reactions in bacterial lipids. *Biochimica et biophysica acta* **70**, 143-151.
- LEE, K. Y., WAHL, R. & BARBU, E. (1956). Contenu en

- bases puriques et pyrimidiques des acides désoxyribonucléiques des bactéries. *Annales de l'Institut Pasteur* **91**, 212–224.
- LEVIN, R. A. (1971). Fatty acids of *Thiobacillus thiooxidans*. *Journal of Bacteriology* **108**, 992–995.
- LEVIN, R. A. (1972). Effect of cultural conditions on the fatty acid composition of *Thiobacillus novellus*. *Journal of Bacteriology* **112**, 903–909.
- LONDON, J. (1963). *Thiobacillus intermedius* nov. sp. A novel type of facultative autotroph. *Archiv für Mikrobiologie* **46**, 329–337.
- LONDON, J. & RITTENBERG, S. C. (1967). *Thiobacillus perometabolis* nov. sp., a non-autotrophic thiobacillus. *Archiv für Mikrobiologie* **59**, 218–225.
- MARKOSYAN, G. E. (1973). A new mixotrophic sulfur bacterium developing in acid media. *Thiobacillus organoparus* sp.n. *Doklady Akademii nauk SSSR* **211**, 1205–1208 (in Russian).
- MARMUR, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *Journal of Molecular Biology* **3**, 208–218.
- MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109–118.
- MIZOGUCHI, T., SATO, T. & OKABE, T. (1976). New sulfur-oxidizing bacteria capable of growing heterotrophically. *Thiobacillus rubellus* nov. sp. and *Thiobacillus delicatus* nov. sp. *Journal of Fermentation Technology* **54**, 181–191.
- MOSS, C. W., SAMUELS, S. B., LIDDLE, J. & MCKINNEY, R. M. (1973). Occurrence of branched-chain hydroxy fatty acids in *Pseudomonas maltophilia*. *Journal of Bacteriology* **114**, 1018–1024.
- MURACA, R. F., WHITTICK, J. S., DAVES, G. D., JR, FRIIS, P. & FOLKERS, K. (1967). Mass spectra of ubiquinones and ubiquinolins. *Journal of the American Chemical Society* **89**, 1505–1508.
- MYERS, P. S. & MILLAR, W. N. (1975). Nonautotrophic *Thiobacillus* in acid mine water. *Applied Microbiology* **30**, 884–886.
- O'LEARY, W. M. (1959). Studies of the utilization of C^{14} -labeled octadecenoic acids by *Lactobacillus arabinosus*. *Journal of Bacteriology* **77**, 367–373.
- SHORT, S. A., WHITE, D. C. & ALEEM, M. I. H. (1969). Phospholipid metabolism in *Ferrobacillus ferrooxidans*. *Journal of Bacteriology* **99**, 142–150.
- SILVERMAN, M. P. & LUNDGREN, D. G. (1959). Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans* I. An improved medium and a harvesting procedure for securing high cell yields. *Journal of Bacteriology* **72**, 642–647.
- STARKEY, R. L. (1935). Isolation of some bacteria which oxidize thiosulfate. *Soil Science* **39**, 197–219.
- TAYLOR, B. F. & HOARE, D. S. (1969). New facultative *Thiobacillus* and a reevaluation of the heterotrophic potential of *Thiobacillus novellus*. *Journal of Bacteriology* **100**, 487–497.
- UCHIDA, K. & MOGI, K. (1972). Cellular fatty acid spectra of *Pediococcus* species in relation to their taxonomy. *Journal of General and Applied Microbiology* **18**, 109–129.
- URAKAMI, T. & KOMAGATA, K. (1979). Cellular fatty acid composition and coenzyme Q system in gram-negative methanol-utilizing bacteria. *Journal of General and Applied Microbiology* **25**, 343–360.
- VISHNIAC, W. V. (1974). Genus *Thiobacillus*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 456–461. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.
- VISHNIAC, W. & SANTER, M. (1957). The thiobacilli. *Bacteriological Reviews* **21**, 195–213.
- WAKSMAN, S. A. (1922). Microorganisms concerned in the oxidation of sulfur in the soil. V. Bacteria oxidizing sulfur under acid and alkaline conditions. *Journal of Bacteriology* **7**, 609–616.
- YAMADA, Y., AIDA, K. & UEMURA, T. (1969). Enzymatic studies on the oxidation of sugar and sugar alcohol V. Ubiquinone of acetic acid bacteria and its relation to classification of genera *Gluconobacter* and *Acetobacter*, especially of the so-called intermediate strains. *Journal of General and Applied Microbiology* **15**, 181–196.
- YAMADA, Y., INOUE, G., TAHARA, Y. & KONDO, K. (1976a). The menaquinone system in the classification of coryneform and nocardioform bacteria and related organisms. *Journal of General and Applied Microbiology* **22**, 203–214.
- YAMADA, Y., NAKAZAYA, E., NAKAKI, A. & KONDO, K. (1976b). Characterization of *Acetobacter xylinum* by ubiquinone system. *Journal of General and Applied Microbiology* **22**, 285–292.