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New Sulfur-oxidizing Bacteria Capable of Growing Heterotrophically, *Thiobacillus rubellus* nov. sp. and *Thiobacillus delicatus* nov. sp.

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Abstract

New thiobacilli, *Thiobacillus rubellus* and *Thiobacillus delicatus*, have been isolated from mine water and characterized. Both microorganisms grew heterotrophically, and their cell yields in yeast extract medium were greatly increased by the addition of thiosulfate. Thiosulfate added to yeast extract medium was oxidized to sulfate through intermediate formation of polythionates. *T. delicatus* oxidized thiosulfate and elemental sulfur under autotrophic conditions, while *T. rubellus* oxidized these sulfur compounds only when organic compounds were added to the medium. The optimum pH range and optimum temperature for initiation of growth of both microorganisms was 5-7 and 30°C, respectively. The G+C content of DNA of *T. rubellus* and *T. delicatus* was 65 and 67 mol %, respectively. The activity of both microorganisms for solubilization of ores was extremely low.

Introduction

During the last 20 years there has been an enormous increase in interest in the new technology of leaching, *i.e.* bacterial leaching of ores.[1~3] The possible application of this leaching process to the recovery of various metals has been confirmed by numerous laboratory and field studies, and the biochemical aspect of the bacteria participating in the solubilization of metal ores have been intensively studied.[4]

Attempts to cultivate such absolutely autotrophic bacteria as *T. thiooxidans*[5] and *T. ferrooxidans*[6,7] under mixotrophic conditions have been made by many workers. However, the isolation of chemolithotrophic bacteria capable of growing heterotrophically has hardly been studied. In the present study, the isolation of new sulfur-oxidizing bacteria capable of growing heterotrophically has been investigated to obtain some knowledge concerning the mechanism of bacterial leaching of ores. The taxonomy of the thiobacilli is also discussed.

Materials and Method

Procedure The isolation of sulfur-oxidizing bacteria was achieved by means of the usual enrichment culture followed by repeated streaking on thiosulfate-agar plates. Unless otherwise stated, the growth studies of the isolated bacteria were carried out as follows: a loopful of cells grown on slants was transferred into 100 ml of the medium in a 500 ml Sakaguchi flask, and incubated at 30°C on a reciprocating shaker. ONM,[8] Colmer,[9] 9K,[10] modified Colmer, YE, YES and bouillion media were used in this study. The compositions

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of the last four media are as follows: modified Colmer medium (NH_4Cl 0.2 g, $MgCl_2 \cdot 6H_2O$ 0.1 g, $CaCl_2 \cdot 2H_2O$ 0.2 g, KH_2PO_4 3 g, $Na_2S_2O_3 \cdot 5H_2O$ 5 g and water 1 *l* (pH 6.0 with NaOH)), YE medium ($NaH_2PO_4 \cdot 2H_2O$ 7.5 g, yeast extract 1 g and water 1 *l* (pH 6.0 with NaOH)), YES medium ($NaH_2PO_4 \cdot 2H_2O$ 7.5 g, yeast extract 1 g, $Na_2S_2O_3 \cdot 5H_2O$ 5 g and water 1 *l* (pH 6.0 with NaOH)), bouillion medium (meat extract 5 g, peptone 10 g and water 1 *l*).

Analysis The amount of sulfate in the reaction mixtures was determined gravimetrically as barium sulfate. The total amount of sulfur in the filtrate obtained in the sulfate analysis was determined gravimetrically as barium sulfate, after oxidation to sulfate with a mixture of potassium chlorate and hydrochloric acid. The amounts of thiosulfate, trithionate and tetrathionate were determined by the analytical method proposed previously.[11] Cell protein was determined by the method of Lowry *et al.* with bovine serum albumin as standard.[12] Gram stain was examined by Hucker modification method.[13] The base composition of DNA was determined by the same procedure as that adopted by Brierley and Brierley[14] except that DNA was hydrolyzed with perchloric acid.[15]

Chemicals Yeast extract was purchased from Daigo Eiyo Chemicals Co., Ltd. Meat extract and peptone were products of Kyokuto Pharmaceutical Co., Ltd. All other chemicals were obtained from commercial sources.

Results and Discussion

Isolation of sulfur-oxidizing bacteria

Isolation of Thiobacillus rubellus Sakaguchi flasks containing 100 ml of Colmer medium were inoculated with approximately 0.1 ml of mine water obtained from Kosaka Mine of Dowa Mining Co., Ltd. and incubated at 30°C under shaking. After 10 days, aliquots of the enrichment cultures were streaked onto thiosulfate-agar plates obtained by solidifying liquid Colmer medium with 2% agar. About 10 days later, reddish colonies became visible on the plates. Pure culture, strain KT-7, was obtained after repeated streaking of well-isolated colonies on thiosulfate-agar plates. Strain KT-7 was then transferred to ONM, Colmer and 9K liquid media to investigate whether or not the isolate had the ability to oxidize elemental sulfur, thiosulfate and ferrous iron, respectively. Growth was not observed at all in elemental sulfur- and ferrous iron-mineral salts media even after 20 days. On the other hand, thiosulfate in Colmer medium was oxidized gradually, showing 15 and 30% oxidation after 6 and 13 days, respectively. However, the turbidity observed was mainly due to the elemental sulfur formed in the decomposition of thiosulfate. When the stock culture was maintained in liquid Colmer medium and transferred every 10 days, the degree of thiosulfate oxidation tended to decrease with increasing number of transfers. On the other hand, strain KT-7 grew well in liquid bouillion and YE media at pH 7. It was also noted that thiosulfate added to bouillion and YE media was oxidized to a great extent. Subsequently, the stock cultures were maintained on bouillion-thiosulfate agar slants and transferred every 2 months. As is evident from the discussion presented later, strain KT-7 seems to be a new species, for which the name *Thiobacillus rubellus* (from Latin, *rubellus,* reddish) is proposed.

Isolation of Thiobacillus delicatus Flasks containing 100 ml of YES medium were inoculated with approximately 0.1 ml of mine water obtained from Tsuchihata Mine of Tanaka Mining Co., Ltd. and incubated at 30°C. After 10 days, a decrease in pH and the formation of sulfate were observed. Aliquots of the enrichment cultures were streaked onto Colmer, YE and YES-agar plates at various pH values. However, colonies of fungi alone developed and attempts to obtain colonies of microorganisms capable of oxidizing thiosulfate were unsuccessful. Variation of the substrate concentration or pH value of the medium gave uniformly negative results. On the other hand, the addition of 1,000 units of an antibiotic, trichomycin, to the liquid YES medium depressed the growth of fungi completely and permitted the development of the colonies of bacterium

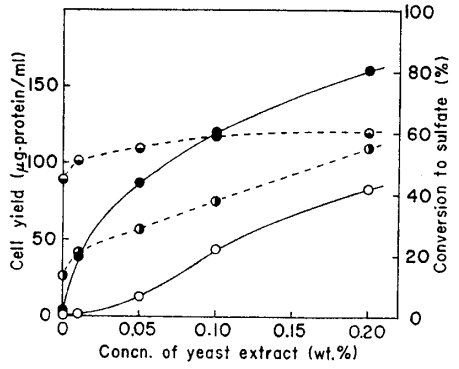

Fig. 1. Effect of the amount of yeast extract added on the growth of *T. rubellus* and *T. delicatus* and on thiosulfate oxidation.
Cell yield: – ○—, *T. rubellus*; --◑--, *T. delicatus*
Conversion to sulfate: —●—, *T. rubellus*; --◒--, *T. delicatus*
Medium, modified Colmer medium with various concentrations of yeast extract; cultivation time, 10 days.

strain TuT-1 on YES-agar plates. Colonies of strain TuT-1 on YES-agar plates were colorless and transparent at first, then turned whitish yellow due to the deposition of elemental sulfur. It was noted that strain TuT-1 perishes within 2 weeks at 30°C, so the stock cultures were maintained on YES-agar slants at about 5°C after the development of colonies and transferred every 2 months. As is evident from the discussion presented later, strain TuT-1 seems to be a new species, for which the name *Thiobacillus delicatus* (from Latin, *delicatus*, delicate) is proposed.

Growth studies

Oxidation of thiosulfate under autotrophic conditions Figure 1 shows the effect of the amount of yeast extract added on the growth of *T. rubellus* and *T. delicatus* and on thiosulfate oxidation. It is clear that *T. rubellus* scarcely grows under autotrophic conditions, and that the degree of thiosulfate oxidation is as low as 2%. On the other hand, the cell yields and the amount of sulfate formed increased with increasing amounts of yeast extract, indicating that organic compounds are essential for the growth of *T. rubellus*. The conversion of *T. rubellus* to autotrophy was attempted by repeated transfer in modified Colmer medium containing decreasing yeast extract concentrations. However, such autotrophic ability as seen in *T. thiooxidans* and *T. ferrooxidans* was not attained by this procedure (Table 1). Accordingly, it may be concluded that *T. rubellus* cannot grow autotrophically with carbon dioxide and thiosulfate as carbon and energy sources, respectively. That *T. rubellus* was isolated using Colmer medium consisting of only inorganic compounds may be attributed to the fact that *T. rubellus* grew initially at the expense of organic compounds present in the mine water, and subsequently on organic compounds in the agar.

As for *T. delicatus*, its cell yield and the amount of sulfate formed also increased with increasing amounts of yeast extract. However, *T. delicatus*, unlike *T. rubellus*, showed noticeable growth without yeast extract (Fig. 1), indicating that *T. delicatus* can grow autotrophically.

*Heterotrophic and mixotrophic growth** Studies of the heterotrophic growth of *T. rubellus* and *T. delicatus* were made by substituting a definite amount of organic compounds for thiosulfate in modified Colmer medium. From the results shown in Table 2, it is clear that both organisms can grow heterotrophically. The mixotrophic growth of *T. rubellus* was investigated by cultivating in modified Colmer medium supplemented with a wide range of organic compounds, the results of which are shown in Table 3. It is clear that various organic compounds such as saccharides, amino acids, alcohols and organic

* The definition of the term "mixotrophic" is that proposed by Rittenberg.[16] Instead of the term "mixotrophic", the term "facultatively autotrophic" is used in *Bergey's Manual of Determinative Bacteriology* (8th ed.)[17] to classify the genus *Thiobacillus*.

Table 1. Repeated transfer of *T. rubellus* in modified Colmer medium containing decreasing yeast extract concentrations.

Transfer No.	Concn. of yeast extract (%)	Cultivation time (day)	Final pH	Cell yield (μg-protein/ml)
1	0.05	10	3.68	10.0
2	0.04	10	5.91	8.5
3	0.03	10	6.83	6.2
4	0.02	10	4.00	2.7
5	0.01	10	3.84	2.7
6	0.01	10	3.72	2.0
7	0.005	10	6.50	2.7
8	0.005	10	6.51	2.2
9	0.001	15	6.44	1.4
10	0	15	6.01	0.5
	0.0001	15	6.00	0.6
	0.0005	15	5.92	0.7
	0.001	15	6.01	1.4
	0.1	10	2.48	28.9
	0.1 (without thiosulfate)	10	6.02	4.9

After 10 or 15 days of cultivation at 30°C on a reciprocal shaker, 2 ml samples were inoculated into fresh medium, and the incubation was repeated.

acids support the growth of *T. rubellus* in a thiosulfate-mineral salts medium, and that thiosulfate is oxidized to sulfate.

Figure 2 shows the effect of thiosulfate concentration on the growth of *T. rubellus* and *T. delicatus* in YES medium. As the results clearly show, *T. rubellus* and *T. delicatus* grow far more favorably under mixotrophic conditions than under heterotrophic conditions. A similar observation has been obtained with *T. intermedius*.[18,19] It may be, therefore, assumed that thiosulfate plays a metabolic role, *i.e.* energy generation, in the presence of yeast extract.[19]

The time cources of thiosulfate oxidation by *T. rubellus* and *T. delicatus* under

Table 2. Heterotrophic growth of *T. rubellus* and *T. delicatus*.

Org. compd. added	(%)	Cell yield (μg-protein/ml)	
		T. rubellus	*T. delicatus*
Yeast extract	(0.1)	10.1	5.6
Fructose	(0.5)	7.9	0.6
Sodium aspartate	(0.5)	—	12.6
Sodium glutamate	(0.5)	11.8	3.4
Glycerol	(0.1)	8.2	1.0
Pyruvic acid	(0.1)	—	10.0

Basal medium: modified Colmer medium without thiosulfate, cultivation time: 10 days.

Table 3. Mixotrophic growth of *T. rubellus*.

Org. compd. added	(%)	Final pH	Cell yield (μg-protein/ml)	Conversion of $S_2O_3^{2-}$ to SO_4^{2-} (%)
Yeast extract	(0.1)	2.71	44.1	60.2
Glucose	(0.5)	3.72	2.3	31.0
Fructose	(0.5)	6.87	9.8	17.8
L-α-Alanine	(0.5)	4.75	2.9	17.4
Sodium aspartate	(0.5)	7.22	6.9	18.3
Sodium glutamate	(0.5)	7.66	14.1	22.2
Glycine	(0.5)	5.77	0.0	0.5
L-Phenylalanine	(0.5)	5.73	0.0	0.2
L-Serine	(0.5)	6.52	6.9	10.6
Glycerol	(0.5)	6.37	5.3	10.3
iso-Propanol	(0.5)	7.13	4.7	7.8
Citric acid	(0.1)	7.55	5.1	5.1
Pyruvic acid	(0.1)	5.51	0.2	0.3
Succinic acid	(0.5)	6.84	11.9	4.4

Basal medium: modified Colmer medium, cultivation time: 10 days.

mixotrophic conditions are shown in Figs. 3 and 4, respectively. The accumulation of a large amount of polythionates was observed. The mechanism of thiosulfate oxidation by these microorganisms will be described in another paper (Sato, T., Mizoguchi, T., Okabe, T.: unpublished).

Effect of the initial pH on the growth of T. rubellus and T. delicatus The relation between cell yield and initial pH of the media were investigated. The results shown in Fig. 5 indicate that both microorganisms grow optimally between pH 5 and 7. Accordingly, it may be said that both *T. rubellus* and *T. delicatus* grow in solutions of higher pH values, compared with such acidophilic bacteria as *T. thiooxidans* and *T. ferrooxidans*,[17] and that the optimal pH values for growth of *T. rubellus* and *T. delicatus* are lower than those for *T. novellus* (7.8–9.0),[20] *T. intermedius* (6.8)[18] and *T. perometabolis* (6.9).[21] The fact that the final pH values are below 5 in some cases shown in Table 2 may be explained by considering that bacteria, once grown, oxidize thiosulfate under conditions which are not suitable as the initial conditions, or that polythionates formed from thiosulfate are decomposed (Sato, T., Mizoguchi, T., Okabe, T.: unpublished).

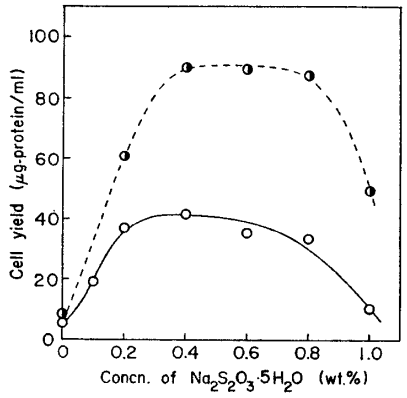

Fig. 2. Effect of thiosulfate concentration on the growth of *T. rubellus* and *T. delicatus*.
—○—, *T. rubellus*; —◑—, *T. delicatus*
Medium, YE medium with various concentrations of thiosulfate; cultivation time, 10 days.

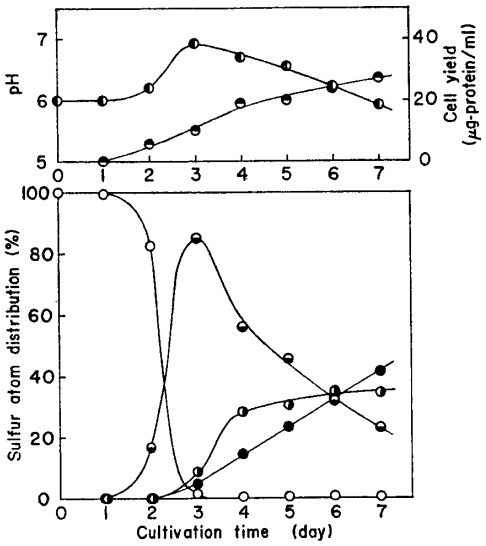

Fig. 3. Time course of thiosulfate oxidation by *T. rubellus*.
◐, pH; ◓, cell yield; ○, $S_2O_3^{2-}$; ◑, $S_3O_6^{2-}$; ◒, $S_4O_6^{2-}$; ●, SO_4^{2-}
A loopful of cells grown on slants was transferred into 200 ml of YES medium, and was incubated at 30°C on a reciprocating shaker. At regular intervals, about 12 ml of the sample solution was taken out and, after the measurement of pH, analysed for sulfur compounds and cell protein by the methods described in the text.

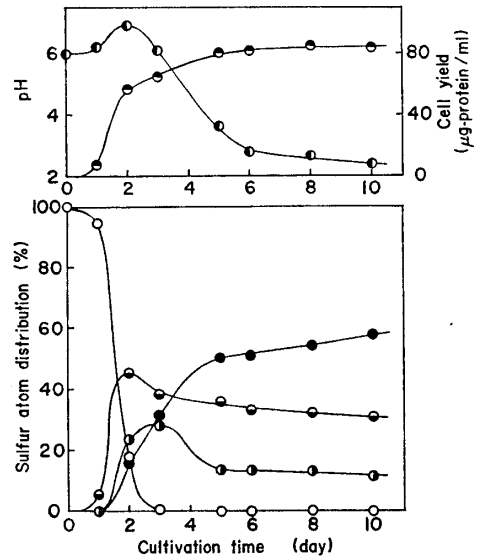

Fig. 4. Time course of thiosulfate oxidation by *T. delicatus*.
Experiment was carried out by the procedure shown in Fig. 3. Symbols are as described in Fig. 3.

Effect of temperature on the growth of T. rubellus and T. delicatus The effect of temperature on the growth of *T. rubellus* and *T. delicatus* was investigated by streaking YES-agar plates with suspensions of the pure cultures and incubating at 20, 25, 30, 35,

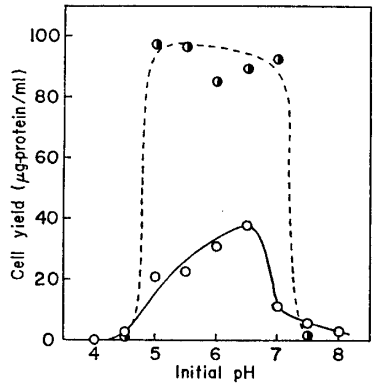

Fig. 5. Effect of the initial pH on the growth of *T. rubellus* and *T. delicatus*.
—○—, *T. rubellus*; --◑--, *T. delicatus*
Medium, YES medium of various pH values; clutivation time, 10 days. pH values are those shown before sterilization, which differed from the pH values shown after inoculation by less than 0.1.

Table 4. Oxidation of elemental sulfur by *T. rubellus* and *T. delicatus*.

Microorganism	Org. compd. added	(%)	Final pH	SO_4^{2-} formed (μg/ml)
T. rubellus	None		5.78	27
	Yeast extract	(0.1)	5.62	123
	Fructose	(0.1)	4.77	90
	Glycerol	(0.1)	3.67	135
T. delicatus	None		3.30	192
	Yeast extract	(0.1)	3.30	682
	Sodium aspartate	(1.0)	3.80	2,200
Control	None		5.78	0

Basal medium: modified Colmer medium without thiosulfate, elemental sulfur added: 1 g, Tween 80 added: 1 ml of 0.25% soln., cultivation time: 10 days.

40 and 45°C for a week. The results indicated that both microorganisms grew slowly at 20°C, but grew well over the temperature range of 25 to 35°C with the optimum at 30°C. The highest temperature for the growth of *T. rubellus* and *T. delicatus* was found to be 35 and 40°C, respectively.

Oxidation of elemental sulfur To investigate their ability to oxidize elemental sulfur, *T. rubellus* and *T. delicatus* were grown in modified Colmer medium from which thiosulfate was omitted, and to which elemental sulfur and organic compounds were added. It is clear from the results shown in Table 4 that, as is in the case of thiosulfate oxidation, *T. rubellus* oxidizes elemental sulfur only when organic compounds are added to the medium. On the other hand, with *T. delicatus* the pH of the medium dropped to 3.3 accompanying the formation of a small amount of sulfate in the absence of organic compounds. The addition of yeast extract and aspartate greatly increased the amount of sulfate formed. Accordingly, it is considered that *T. delicatus* possesses the ability to oxidize elemental sulfur not only under mixotrophic conditions but also under autotrophic conditions. However, the oxidation activity of *T. rubellus* and *T. delicatus* toward elemental sulfur is extremely low, considering that *T. thiooxidans* oxidizes elemental sulfur, resulting in a sulfate concentration of about 10 mg/ml.[22]

Solubilization of ores The leaching of ores was performed. As is evident from the results shown in Fig. 6, the amount of sulfur released from "Kurokō" (black ore)* increased with increasing leaching time in the presence of the isolated bacteria. However, it is not clear from what kinds of minerals sulfur is released. On the other hand, the amount of copper released was as low as 2–9 μg/g-ore, and was independent of leaching time and the presence of bacteria. The degree of dissolution of copper and of sulfur from chalcopyrite and "Keikō" (sliceous ore)* were very small regardless of the presence of bacteria. Consequently, it may be concluded that the activities of *T. rubellus* and of *T. delicatus* for solubilization of ores are extremely small, compared with those of such acidophilic bacteria as *T. thiooxidans* and *T. ferrooxidnas*.[1~3,23,24]

It has been demonstrated by many studies that the cell yields obtained under mixotrophic or heterotrophic conditions are higher than those obtained under autotrophic conditions, while cells harvested from media supplemented with organic compounds

* "Kurokō" (black ore) and "Keikō" (siliceous ore) together with "Ōkō" (yellow ore) are the most typical ore types of the so-called "Kurokō deposits". "Kurokō" is rich in sphalerite, galena and barite with some tetrahedrite, pyrite and chalcopyrite. "Keikō" is highly siliceous ore with some chalcopyrite and pyrite.

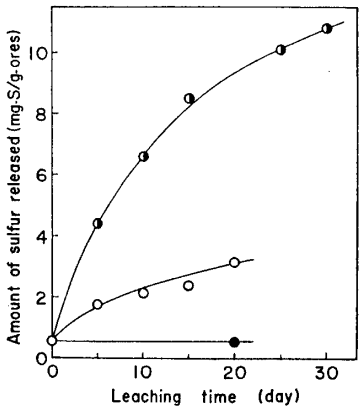

Fig. 6. Leaching of "Kurokō" in the presence of *T. rubellus* and *T. delicatus*.
○, *T. rubellus*; ◑, *T. delicatus*; ●, control
One hundred ml of YE medium was put into a 500 ml Sakaguchi flask together with 1 g of "Kurokō" (<100 mesh). After sterilization for 15 min at 120°C, the contents were inoculated with 5 ml of YE medium which had previously been incubated with *T. rubellus* or *T. delicatus*, and was then shaken reciprocally at 30°C.
Composition of "Kurokō" used (%): Cu 10.3, Fe 5.57, Pb 1.26, Zn 4.46, S 14.35, SiO_2 2.81 and $BaSO_4$ 60.04.

show a reduced rate of oxidation of inorganic compounds.[7,25,26] However, the present results indicate the possibility of finding various types of bacteria in mine water, and that there might exist bacteria having an enhanced growth rate and leaching activity in the presence of organic compounds.

Classification It has been recognized that heterotrophic organisms oxidize thiosulfate quantitatively to tetrathionate without obtaining energy from this process.[27] *T. rubellus* and *T. delicatus*, however, may be assigned to thiobacilli[17] by considering the following aspects: 1) both microorganisms are Gram-negative rods capable of obtaining energy by oxidizing thiosulfate to sulfate, 2) their base composition of DNA is close to that of *T. novellus* and *T. denitrificans* (see Table 5).[28]

Table 5. Base composition of DNA of *T. rubellus*, *T. delicatus* and other thiobacilli.[28]

Species	Strain	G+C (mol %)	Group[28]
*T. rubellus**	KT-7	65±3	
*T. delicatus**	TuT-1	67±3	
*T. trautweini***	NCIB 9549	66	
T. novellus	NCIB 9113	68	
	NCIB 8093	66	
T. denitrificans	Baas-Becking (Trudinger)	64	(1)
T. thioparus	NCIB 8349	66	
	NCIB 8370	62	
*T. thiocyanoxidans***	NCIB 5177	63	
T. neapolitanus	Baas-Becking (Trudinger)	56	
T. ferrooxidans	(Aleem)	57	(2)
T. thiooxidans	NCIB 9112	52	
	NCIB 8085	52	
*T. concretivorus***	NCIB 9514	51	(3)
	1P	51	
	2P	52	

* Both microorganisms were grown in YES medium.
** These microorganisms are not included in *Bergey's Manual of Determinative Bacteriology* (8th ed.).

T. rubellus cannot grow autotrophically, but can grow heterotrophically. This organism oxidizes thiosulfate only in the presence of organic compounds. These characteristics of *T. rubellus* are in good agreement with the definition of chemolithotrophic heterotrophs proposed by Rittenberg.[16,25] *T. perometabolis*[21] was considered the only chemolithotrophic heterotroph that can oxidize low valence sulfur compounds.[16,17,25] *T. rubellus*, however, seems to be a new chemolithotrophic heterotroph distinct from *T. perometabolis*, since *T. rubellus* forms reddish colonies and grows in a mineral salts medium supplemented with such single carbon sources as glycerol and glutamate.

On the other hand, *T. delicatus* can grow not only heterotrophically but also autotrophically, indicating that *T. delicatus* belongs to mixotrophs. Only two thiobacilli, *i.e.* *T. novellus*[20] and *T. intermedius*,[18] have been assigned to mixotrophs.[16,17] The base composition of DNA of *T. delicatus* as well as *T. rubellus* resembled that of *T. novellus* (Table 5). *T. delicatus*, however, may be differentiated from *T. novellus* by the following aspects: 1) *T. delicatus* oxidizes elemental sulfur, even if its extent is low, 2) the optimum pH range for growth of *T. delicatus* (5–7) is lower than that of *T. novellus* (7.8–9.0),[20] 3) the pH seldom drops below 5.8 in the oxidation of thiosulfate by *T. novellus*,[20] 4) the phenomenon of higher cell yield under mixotrophic conditions has not been reported for *T. novellus*. *T. delicatus* is distinguished from *T. intermedius* in that *T. delicatus* can grow on a wide range of single carbon compounds, and that flagella are not seen in *T. delicatus*. Thiobacillus A2, a new mixotrophic bacterium described by Taylor and Hoare,[29] may be differentiated from *T. delicatus*, since Thiobacillus A2 is very similar to *T. novellus* except that Thiobacillus A2 utilizes various kinds of organic compounds.

Description of *Thiobacillus rubellus* nov. sp. Cells are rod-shaped, usually occuring singly, and rarely in pairs, 1.0–1.6 μm by 1.7–3.0 μm, Gram-negative, non-spore forming and motile by means of a single polar flagellum (Fig. 7). Colonies on 0.1% yeast extract —0.5% thiosulfate agar are approximately 1 mm in diameter with smooth outer edge, and are tinged with red. The center of the colonies become dark after about 2 weeks incubation. Cells are not capable of autotrophic growth. Good growth occurs in thiosulfate mineral salts medium supplemented with such organic compounds as yeast extract, glycerol, glutamate, fructose and aspartate. Heterotrophic growth occurs in yeast extract medium and in mineral salts medium supplemented with single carbon sources, *e.g.* glycerol, glutamate and fructose. The cell yield in yeast extract medium is greatly increased by the addition of thiosulfate. Its oxidation activity towards elemental sulfur is extremely low even if organic compounds are present. Optimal growth conditions are 30°C and between pH 5 and 7. Marginal growth occurs at 35°C. The G+C content of DNA is 65$\pm$3 mol %.

Description of *Thiobacillus delicatus* nov. sp. Cells are rod-shaped, usually

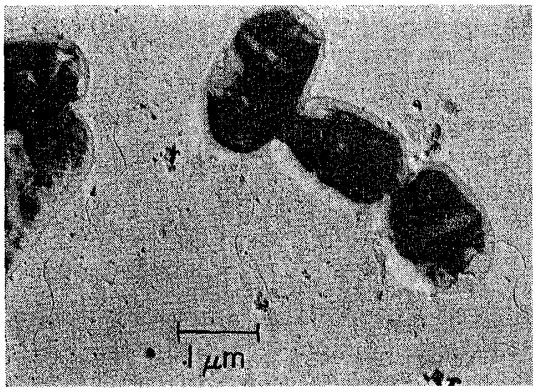

Fig. 7. Electron micrograph of *T. rubellus* grown in YES medium.

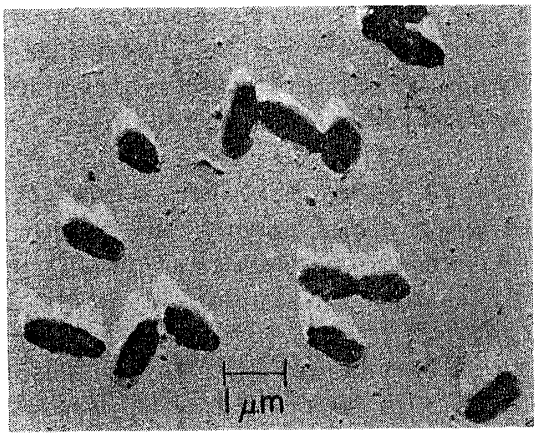

Fig. 8. Electron micrograph of *T. delicatus* grown in YES medium.

occurring singly, and rarely in paris, 0.45–0.50 μm by 0.7–1.3 μm, Gram-negative, non-spore forming and nonmotile (Fig. 8). Colonies on 0.1% yeast extract —0.5% thiosulfate agar are approximately 1 mm in diameter with smooth outer edge. The colonies are colorless and transparent at first, and then turn whitish yellow due to the deposition of elemental sulfur. Cells are capable of growing autotrophically in thiosulfate mineral salts medium. In yeast extract medium and in mineral salts medium supplemented with single carbon sources, *e.g.* glutamate, pyruvate and aspartate, heterotrophic growth occurs. The cell yield in yeast extract medium is greatly increased by the addition of thiosulfate. The oxidation activity towards elemental sulfur is very low, compared with that of such acidophilic thiobacilli as *T. thiooxidans* and *T. ferrooxidans*. The rate of oxidation of elemental sulfur is enhanced by the addition of organic compounds, *e.g.* yeast extract and aspartate. Optimal growth conditions are 30°C and between pH 5 and 7. Marginal growth occurs at 40°C. The G+C content of DNA is 67±3 mol %.

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