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MORPHOLOGICAL, PHYSIOLOGICAL, AND CHEMOTAXONOMICAL CHARACTERISTICS OF IRON-AND SULFUR-OXIDIZING BACTERIA ISOLATED
FROM ACID MINE DRAINAGE WATERS

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Two strains, Fe 1 and Fe2, of iron-oxidizing bacteria from acid drainage water at the Matsuo mine showed dissimilar characteristics of red-brown

colonies on FeSO4-9K silica gel plates. The Fe l colonies were large and irregular. The Fe2 colonies were minute and circular. These bacteria, identified as Thiobacillus ferrooxidans on the basis of their physiological and chemotaxonomical properties (fatty acid composition, ubiquinone type, andl DNA base composition), were different strains. Fe2 differed from Fe l in its extremely low oxidizing activity of sulfur and thiosulfate. Two strains, S2 and S3, of sulfur-oxidizing bacteria were separately isolated on Na2SzO3-9K silica gel plates and agar plates, respectively. However, S2 and S3 formed pale brown and pale yellow colonies on the silica gel and agar plates, respectively. Since these two strains were identical in their properties, they were judged to be quite similar or the same strain. From
their morphological, physiological, and chemotaxonomical properties,
they were identified as Thiobacillus thiooxidans. The oxidation characteristics of both sulfur and thiosulfate by the strains of iron- and sulfur-oxidizing bacteria were also investigated.

The sulfur-oxidizing bacterium Thiobacillus thiooxidans was first isolated in 1921 from soil (28). Twenty-six years later Colmer and Hinkle reported the first isolation of Thiobacillus ferrooxidans responsible for the oxidation of iron and inorganic sulfur compounds from the acid mine drainage of bituminous coal mines (1). Since then, the iron- and sulfur-oxidizing Thiobacillus species were shown to occur widely in nature especially where oxidisable sulfur and iron are

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abundant (11). T. ferrooxidans and T, thiooxidans have continued to draw much interest because of their unique physiological characters (e.g. autotrophilism and acidophilism), environmental impact (e.g. acid and metal pollution), and commercial value (e.g. metal recovery by bacterial leaching and desulfurization of coal). However, current knowledge of T, ferrooxidans and T. thiooxidans is based on
a study of relatively few strains, as they are rather difficult to produce in pure culture and are troublesome to maintain. Extensive and systematic collections of these species from diverse localities and habitats in the world are very important for better understanding their genetic, physiological, and taxonomical properties (8). We studied the ecological aspects of acidophilic chemolithotrophic iron- and sulfur-oxidizing bacteria at the abandoned Matsuo sulfur and iron sulfide (pyrite) mine areas (21, 22, 24) and the leaching of pyrite ores by the bacteria (25-27). We found that iron- and sulfur-oxidizing bacteria were ubiquitously distributed in both acid mine waters and pyrite ores, and were responsible for the rapid oxidation of iron and sulfur and the degradation of the ores.
However, no detailed information has been obtained about the biology of the bacteria living in the mine areas. In this investigation we isolated and characterized the iron- and sulfur-oxidizing bacteria (T. ferrooxidans and T. thiooxidans) from the mine areas. The presence of different strains of iron-oxidizing bacteria is re-ported.

MATERIALS AND METHODS

Study area and sampling. The abandoned Matsuo sulfur and iron sulfide mine
is on the Hachimantai volcano in Iwate Prefecture. Many small creeks and a large stream of acid mine water are discharged from the mine area and pour directly into the River Akagawa, meaning red river. Properties of the acid mine waters and the acid-polluted river water and sampling stations were shown in previous reports (22, 24). Water samples were collected in sterilized 0.5-1 Ehrlenmyer flasks and brought to the laboratory for analyses within 5 h after sampling. The sampling period was from 1976 until 1980.
Bacteria. Thiobacillus ferrooxidans NCIB 8455 was used as a type strain of iron-oxidizing bacteria.
Media. Distilled water was used to prepare all media. The pH of the media was adjusted by adding H2SO4 or NaOH. Liquid and solid cultures were incubat-ed at 30 and 25°C, respectively, unless otherwise noted. The basal salts medium of 9K reported by Silverman and Lundgren (18) was used throughout this study. It was composed of 3.0 g (NH4)2504, 0.1 g KC1, 0.5 g K2HPO4, 0.5 g MgSO4.7H2O, and 0.01 g Ca(N03)2 per liter of deionized water. FeSO4-9K (pH 3.0), S°-9K (pH 3.0), and Na2S203-9K (pH 5.0) media were prepared by supplementing the basal salts medium with 2% (w/v) FeSO4.7H2O, 2% (w/v) elemental sulfur, and 1 % (w/v) Na2S203.5H2O, respectively. FeSO4 and Na2S203 solutions were pre-pared separately and sterilized by filtration through a 0.2 ,um membrane filter (Toyo

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Roshi Co.). Powdered elemental sulfur was sterilized by autoclaving at 120°C for 30 min. FeS04-9K silica gel and Na2S203-9K agar (Difco Bacto agar, Difco Labo-ratories, Detroit) plates were prepared according to the procedures described previously (21, 23). The same procedures were also used to prepare Na2S203-9K silica gel plates. Nutrient broth was composed of 10 g polypeptone, 10 g meat ex-tract, and 5 g NaCI per liter of deionized water, and adjusted to pH 3. Cultivation. A 50-ml liquid medium prepared in a 500-ml flat-bottom culture flask was used in this study, unless otherwise noted. One ml of cell suspension was usually inoculated into the 50 ml liquid medium. Shaking cultures were carried out on a reciprocal shaker operating at 120 rpm. A large-scale culture of iron-oxidizing bacteria was made using 3 l of Fe504-9K medium in a 3-l flat-bottom flask under forced aeration at 25°C for 3-4 days. For mass culture of sulfur-oxidizing bacteria, many 500-ml flat-bottom flasks containing 100 ml of S°-9K liquid medium in each were shaken for a week. For plating out, 0.1 ml of cell suspension or water sample was spread on a silica gel or agar plate with a bent glass rod. The inoculated plates were incubated in a sealed polyvinyl-bag to keep the medium from drying. Oxygen requirement was examined by cultivating the bacteria in GasPak Anaerobic Systems (BBL, Cockeysville, Md.). The effect of temperature on growth was examined in the range of 3 to 50°C using a Temperature Gradient Incubator TN-3 (Toyo Scientific Co.). The effect of pH on growth and activity (utilization of substrates as an energy source) was examined in the range of pH from 1 to 7. Glucose utilization was verified according to the method of Shafia and Wilkinson (17), in which 9K salts medium (pH 3.0) supplemented with glucose (1%) and yeast extract (0.01%) was used. Utilization of (NH4)2504, NH4C1, and KN03 as an inorganic nitrogen source (640 mg N/l) was examined using nitrogen-free Fe504-9K and S°-9K media. Utilization of organic nitrogen compounds (0.1 g/l), such as, peptone, yeast extract, and glutamic acid, was also examined as nitrogen sources.
Cell harvest. After liquid cultivation of iron- or sulfur-oxidizing bacteria, followed by centrifugation at 1,500 x g for 10 min to remove iron precipitates or residual sulfur particles, the bacterial cells in the supernatants were harvested by centrifuging at 12,000 x g for 10 min. The resulting cell pellet was washed three times with sterilized acid water (pH 1.5) and then twice with sterilized water, and finally resuspended in a small volume of sterilized water to give the original cell suspensions used as inocula.
Cell count. Total bacterial numbers were counted with a bacterial counting chamber (Erma Optics Co., Tokyo) under a phase-contrast microscope. Viable numbers were estimated by spreading appropriate dilutions on the solid medium. Analyses. Sulfate and soluble iron (total and ferrous) were determined by the barium sulfate turbidity (4) and a,a'-dipyridyl (6) methods, respectively. The amounts of sulfate and iron were calculated in mg/l. The degree of iron oxidation was expressed as the ratio of ferric to total iron (Fe3+/2Fe; %). Thiosulfate and

glucose were determined by the iodometric titration (19) and phenol-sulfate (5)

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methods, respectively, and were calculated in mg/ml. pH was measured with a glass electrode pH meter.
Light microscopy. The sizes and shapes of the bacterial cells stained with a dilute Ziehl carbolic fuchsin were observed under a light microscope. Gram staining was carried out by a modification of Hucker's method (7).
Electron microscopy. The bacterial cells in liquid cultures were harvested and washed according to the procedures described above. The cell pellets were resuspended and fixed in 2% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for more than 2 h at 4°C. The fixed cells were mounted on copper grids, evaporated with Cr, and examined with a Hitachi model HU-125E transmission electron microscope.
DNA base composition. DNA was prepared from the cells of late exponential liquid cultures according to the method of Marmur (12). The G + C content of DNA (mol%) was obtained by the melting temperature (Tm) method of Marmur and Doty (13) as modified by De Ley (3), using a Beckman DU-8 spectrophotometer (Beckman-Japan Co., Tokyo). The DNA of Escherichia coli K12 was used as a reference.

Quinone system. Cells were collected during the early stationary growth and freeze-dried. Quinones were extracted from the lyophilized cells and purified by thin-layer chromatography using a silica-gel plate (GF 254; E. Merck) as described by Katayama-Fujimura et al. (10). The purified ubiquinone system was analyzed by a Shimadzu High-Performance Liquid Chromatograph LC-3A (Shimadzu Seisakusho Ltd., Kyoto) fitted with a Dupont Zorbax-ODS column (4.6 mm x 250 mm) and methanol-iso-propylether (3: 1, v/v) (1 ml/min) as the mobile phase. The quinones were monitored at 275 nm and quantified using a Shimadzu Chromatopac CR-3A.

Cellular fatty acid composition. Cells were harvested during an early stationary growth, and lyophilized. Fatty acids of the lyophilized cells were methylated and extracted by the method of Miyagawa et al. (14). Fatty acid methyl esters were analyzed using a Shimadzu gas-liquid chromatograph (GLC) GC-8APF with a chromatograph-data processor C-R6A. The GLC apparatus was equipped with a fused silica capillary column (0.25 mm x 30 m) with SPB-1 (Supelco Japan Ltd., Tokyo). Fatty acids were identified by comparing the retention time of their methyl esters with those of authentic standard fatty acid methyl esters (Supelco Bacterial Acid Methyl Esters CP-Mix). Hydrogenation was used to identify unsaturated and cyclopropane fatty acids. The fatty acids were identified by a Hitachi GC-MS system M 2000 equipped with a HP-5 capillary column (0.35 mm x 25 m) coated with methyl-silicone (Hewlett Packard Co., Calif.)

RESULTS

Colony appearances and isolation
Appropriate dilutions of acid mine waters (pH 1.5-1.7, Fee + 1,000 mg/l)

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discharged from the mine drift were applied to plating out on Na2S203-9K silica gel and agar media, and on FeSO4-9K silica gel medium. After 3 weeks of incubation, colonies developed on the plates were observed and isolated.
Red-brown colonies of iron-oxidizing bacteria grown on FeSO4-silica gel plates were divided into two types depending upon colony appearances. One type (Fel) was relatively large and irregular, and the other (Fe2) was minute and circular. Each type of the colony was cultivated in FeSO4-9K liquid medium for a week. After the media became red-brown, the cultures were again inoculated on FeSO4-9K silica gel plates. Such ordinary purification procedures were repeated several times, and finally pure cultures of the two types were obtained. Colonies of strain Fe l (Fig. la) were large (l-4 mm) and irregular (auriculate with unequal projections). Colonies of strain Fe2 (Fig, l b) were very minute (0.2-0.7 mm), circular, and entire. Colonies of Fe2 were morphologically similar to those of the type strain T. ferrooxidans N CIB 8455
Three types of colonies (Sl, S2, and S3) were observed on Na2S203-9K solid medium: creamy white (Sl) and pale brown (S2) colonies on the silica gel medium, and pale yellow (S3) on the agar medium. The creamy white colonies (Sl) on the silica gel medium were circular, entire, convex or pulvinate, smooth, glistening, and 0.5-2 mm in diameter. Pale brown colonies (S2) were circular, entire or undulate, convex, smooth, glistening, and 2-4 mm in diameter. Pale yellow colonies (S3) on the agar medium were circular, entire, convex, smooth, and 2-3 mm in diameter. Each of colonies Sl, S2, and S3 was inoculated and cultivated in both Na2S203- and S°-9K media. The S2 and S3 colonies grew vigorously in these liquid media. On the other hand, the strain S2 formed pale yellow colonies on the agar plates. In parallel experiments, strain S3 formed pale brown colonies on silica gel plates. Apparently the difference of colony morphology between strains S2 and S3 resulted from the kinds of the solidifying agents used. However, S 1 colonies failed to grow in either Na2S203-9K and S°-9K media or FeSO4-9K medium.

Fig. 1. Colonial appearance of iron-oxidizing bacteria strains Fel
growing on FeSO4-9K silica gel plates.

(a) and Fe2 (b)

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Cell morphology and some properties
Cells of S 1 colonies in electron microscopic preparations were single, polar flagellated, and rod-shaped. The creamy white S 1 colonies were easily dissolved in CS2, indicating the formation of elemental sulfur crystals outside the cells from thiosulfate.

Strains of sulfur-oxidizing bacteria (S2 and S3) were gram negative, strictly aerobic, single polar flagellated, and rod shaped (0.4-0.7 x 0.9-2.2 µm). Strains of iron-oxidizing bacteria (Fe l and Fe2) were also gram negative, strictly aerobic, and rod shaped (0.4-0.8 x 0.8-2.3 µm). Temperatures for growth and sulfur oxidation of strains S2 and S3 were in the range of 5-37°C (optimum temperature: 25-33°C). No growth and sulfur oxidation occurred at about 5°C and above 40°C. Temperatures for growth and iron oxidation of Fe 1 and Fe2 were in the range of 5-43°C (optimum temperature: 25-35°C). Growth and iron oxidation occurred even at about 5°C but not above about 45°C. Ammonium-nitrogen was the most suitable source of nitrogen for all of the strains. Nitrate-nitrogen, peptone, yeast extract, and glutamic acid scarcely affected their growth and oxidation of the substrates. No growth of all the strains occurred in nutrient broth at acid conditions.

Oxidation of iron

The effect of pH on the growth and iron oxidation was studied in three strains
of iron-oxidizing bacteria (Fel, Fe2, and T. ferrooxidans NCIB 8455). As shown in the representative results (Fig. 2a), both growth and iron oxidation of all strains occurred at narrow pH ranges (pH 1-4). Their optimum pH was 2-3. The progressive

Fig. 2. Effects of pH on the growth of iron-oxidizing bacteria (Fe!) and the oxidation
of iron, and the progressive changes in its growth and iron oxidation.
(a): FeSO4-9K media adjusted to pH 0.5-5 were used and cultivated for 41 h. (b):
FeSO4-9K medium (pH 2) was used. •: iron oxidation, o: cell number. Dotted lines in
this and subsequent figures indicate the initial cell numbers added.

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changes in their iron oxidation and growth were almost parallel until iron was completely oxidized (Fig. 2b). The specific growth rate (µ) (h') and specific iron oxidation rate (h') were 0.07 and 0.11 (Fe 1), 0.05 and 0.10 (Fe2), and 0.06 and 0.10 (T, ferrooxidans NCIB 8455).

Oxidation of sulfur

Figure 3 shows representative results of the growth of iron- and sulfur-oxidizing bacteria (Fe 1, Fe2, and S2) and sulfur oxidation, and the effect of pH on the oxidation. As shown in Fig. 3a, strains of sulfur-oxidizing bacteria had very high sulfur oxidation activities in the range of pH 1-6 and an optimum pH of 3-5. The media pH decreased finally to 0.5-0.7 because of active sulfur oxidation by the sulfur-oxidizing thiobacilli. Strain Fel and also T. ferrooxidans NCIB 8455 showed relatively high sulfur-oxidizing activity with an optimum at pH 4-5. With the iron-oxidizing thiobacilli, the media pH also decreased finally to about 0.5 due to prolonged sulfur oxidation. The values of µ were 0.08 (Fe 1), 0.07 (T. ferrooxidans NCIB 8455), 0.09 (S2), and 0.07 (S3). However, the sulfur-oxidizing activity of strain Fe2 was extremely low. Only feeble sulfur oxidation occurred in the range of pH 4-6 after 7 days cultivation with a large Inoculum (4 x 109/ml) (Fig. 3a). A very small increase of sulfate (300 mg/l) was produced by Fe2, with no detectable increase in bacterial numbers even after 12 days of incubation (Fig. 3b). And adding a trace metal solution (Zn2 +, Mn2 +, Cue +, Cot +, B3 +, or Mob +) and surface active agent (Tween 80) did no increase the sulfur oxidation.

Fig. 3. Effects of pH on sulfur oxidation by iron- and sulfur-oxidizing bacteria
(Fe!, Fe2, and S2) and progressive changes in their growth and oxidation of the substrate.
(a): S°-9K media adjusted to pH 1-7 were used. Inoculum size: 1.6 x 108/ml (S2) (o,
1-day culture; •, 3-day culture); 2 x 108/ml (Fe 1) (A, 7-day culture); 2 x 109/ml (Fe!) (o,
7-day culture); 4 x 109/ml (Fe2) (t, 7-day culture). (b): S°-9K medium (pH 5) was used. Inoculum size: 8 x 10'/ml (S2) (•, o); 2 x 109/ml (Fe!) (A, o); 4 x 109/ml (Fe2) (•, o). Closed and open symbols indicate cell number and sulfate, respectively.

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Oxidation of thiosulfate

Strains S2 and S3 of sulfur-oxidizing bacteria oxidized much thiosulfate but
not iron. The pH ranges for their apparent thiosulfate oxidation was 4.3-5.5. The optimum was pH 4.8-5.0 (Fig. 4). Strains of iron-oxidizing bacteria (Fe l and T. ferrooxidans NCIB 8455) also had high thiosulfate-oxidizing activities in the pH range of 4.2-5.6 and the optimum pH was 4.6-5.0 (Fig. 5). The values of t were 0.06 (S2), 0.05 (S3), 0.05 (Fel), and 0.04 (T. ferrooxidans NCIB 8455). As shown in Figs. 4 and 5, the pH values and thiosulfate consumption of the media tended to increase gradually in the acid pH ranges when the thiosulfate was not oxidized, suggesting chemical decomposition of the substrate in the acid condition..
There was a difference in the thiosulfate oxidation between the sulfur- and

iron-oxidizing bacteria (Fig. 6). In the sulfur-oxidizing bacteria, both the bacterial growth and pH decrease (sulfate increase) stopped just when thiosulfate was completely consumed. But, with iron-oxidizing bacteria, the cell numbers increased along with a pH decrease (sulfate increase) even after complete absence of the substrate. This shows the differences in the thiosulfate-metabolizing system of iron- and sulfur-oxidizing bacteria.
As shown in Fig. 7, the thiosulfate oxidation by strain Fe2 was extremely low. But the oxidation by strain Fe l was very fast with only a small inoculum (1 x 10'/ml) (Fig. 5). However, with a large bacterial inocula (ca. 109/ml), very weak and unstable oxidation of the substrate occurred after a prolonged incubation (Fig. 7b).

Fig. 4. Effects of pH on growth of sulfur-oxidizing bacteria (S2) and oxidation of
thiosulfate.
(a): Na2S203-9K media adjusted to pH 2.3-6.8 were used. Inoculum size: 1 x 10'/ml.
A: cell number (2-day culture), •: thiosulfate (2-day culture), o: thiosulfate (3-day culture).
(b): The same experiment as in (a). •: 2-day culture, o: 3-day culture.

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Glucose utilization

To determine whether the strains of iron-oxidizing bacteria (Fel, Fe2, and T. ferrooxidans NCIB 8455) grow heterotrophically, these bacteria, after a brief previous adaptation to glucose in FeSO4-9K medium, were cultivated in 9K salts

Fig. 5. Effects of pH on growth of iron-oxidizing bacteria (Fe!) and oxidation of
thiosulfate.
(a): Na2S203-9K media adjusted to pH 2.3-6.8 were used. Inoculum size: 1 x 10'/ml.
A: cell number (3-day culture), •: thiosulfate (3-day culture), o: thiosulfate (5-day culture).
(b): The same experiment as in (a). •: 3-day culture. o: 5-day culture.

Fig. 6. Progressive changes in growth of iron- and sulfur-oxidizing bacteria (Fe! and S2) on thiosulfate and its oxidation.
Na2S203-9K medium (pH 5) was used
Fe!: o, cell number; o, thiosulfate; o, pH.

S2: •, cell number; A, thiosulfate; •, pH.

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medium with 1 % (w/v) glucose. As shown in Table 1,
glucose, indicating their obligate chemolithotrophy.

none of the strains grew on

Chemotaxonomical characteristics

As shown in Table 2 with some published data, all the strains (Fe 1, Fe2, S2, and S3) of iron- and sulfur-oxidizing bacteria contained ubiquinone Q-8 as a major component. The DNA G + C contents of strains Fe l and Fe2 were 56-57 mol%, whereas those of the strains S2 and S3 were 51-52 mol%. Strains Fel and Fe2 had C16: 0, C16:1, and C18:: 1 as major non-hydroxylated fatty acids. Strains S2 and S3 had C12: 0, C16:1, C18:1, and C19cyc as major non-hydroxylated fatty acids. All the strains had 3-OH C14: 0 as hydroxy fatty acids. The cellular fatty acid composition of strains Fel (Fe2) and S2 (S3) were almost the same as those of the type strains of T, ferrooxidans and T, thiooxidans, respectively.

Fig. 7. Effects of pH on the oxidation of thiosulfate by iron-oxidizing bacteria (Fe2)
and progressive changes in its oxidation.
(a): Na2S203-9K media adjusted to pH 3.5-6.8 were used and cultivated for 10 days.
Inoculum size: 1 x 109/ml. •: pH, o: thiosulfate. (b) Inoculum size: 4 x 109/ml. • and A:
pH, o and o: thiosulfate. Data of replicate flasks were given here.

Table 1. Utilization of glucose by the strains of iron-oxidizing bacteria.

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DISCUSSION

All the strains (Fe 1, Fe2, S2, and S3) isolated from the Matsuo mine were obligately autotrophic, aerobic, acidophilic, gram negative, non-spore forming, and rod shaped. Strains 52 and 53 are quite similar in properties and were identified as T. thiooxidans. Strains Fe l and Fe2 belong to T. ferrooxidans. Fe l has often been used in our studies (25-27). However, strains Fel and Fe2 are different in their ability to oxidize sulfur and thiosulfate. Strain Fe2 oxidizes the substrates very weakly. It is fortuitous that the two strains Fe l and Fe2 may be different among the strains of T. ferrooxidans. The phenotypical characteristics of T. ferrooxidans or T. thiooxidans are too few to classify and identify the groups of numerous bacterial strains solely by them. A. P. Harrison (8) studied the genomic and physiological diversity among 23 strains of T. ferrooxidans, and showed that the strains have a wide range of G + C content (53-65 mol%) and diverge into 7 different DNA homology groups. His study demonstrates genetic differences at the strain level of T. ferrooxidans and T. thiooxidans, and also indicates the importance of studying systematically the genetic and physiological characteristics of a large collection of strains isolated from various habitats.

It was difficult to determine exactly the ratios of Fel and Fe2 colonies developed
on ferrous iron solid medium, but the Fe l colonies were rather dominant in acid mine drainage waters. It is significant that different strains of iron-oxidizing bacteria are coexistent in the same water sample. Between the two kinds of colonies (S 1 and 52) developed on thiosulfate silica gel medium, S2 was easily isolated and identified as a typical strain of T, thiooxidans, but S1 did not grow in pure cultures with either sulfur, thiosulfate, or iron as a sole energy source. This signifies the importance of the coexistence of S 1 with S2 for the growth of S 1 or the presence of unknown growth factors. The counts of S 1 and S2 were almost equal, 102/ml in acid mine waters and 106/g in pyrite ores. Considering the presence of a large number of S l in the mine areas, it is worthwhile to try to isolate S 1 and investigate its characteristics.

As indicated in Figs. 4 and 5, the thiobacilli commence to grow on thiosulfate only in the range of pH 4.2 to 5.6 but in sulfur-containing medium the pH range extends widely from 1 to 6. These results are supported by the findings of previous workers (2, 9). Tuovinen and Kelly (20) reported also the narrow starting pH range (pH 3.6-4.7) for growth on thiosulfate, which is smaller by about one pH unit than the present results.
It is noteworthy that distinct differences in the pH ranges for the oxidation of thiosulfate, sulfur, and ferrous iron by the acidophilic thiobacilli are related to the stability of the substrates for energy in the acid pH range. Sulfur is extremely stable chemically and can be utilized as an energy source over a wide acid pH range (pH 1-6). Ferrous iron, serving as a favorite substrate for iron-oxidizing bacteria, is stable and almost not auto-oxidizable in strong acid conditions below pH 4.0. But the favorite pH for thiosulfate oxidation by the thiobacilli living below pH ca.

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6 are in more limited and milder acid conditions (pH 4.2-5.6) than that for iron oxidation, possibly because thiosulfate is unstable and spontaneously decomposable in the acid conditions below pH 4.
Iron- and sulfur-oxidizing thiobacilli fail to grow on thiosulfate in acid condition below pH 4.2. The fate of thiosulfate in those acid conditions is complicated and obscure, but there is evidence that tetrathionate, trithionate, and related compounds (sulfur, sulfide, sulfite, bisulfite, various polythionic acids) are formed during thiosulfate oxidation by the thiobacilli (15, 16, 20). It is possible that thiosulfate and its related products in solution at pH 4.2 and below, probably including HSz03 - and S03 -, inhibit either thiosulfate uptake or metabolism by the thiobacilli (20). Strains Fe 1, Fe2, S2, and S3 have been given laboratory strain names, AIU
001, 002, 101, and 102, respectively, and also have been deposited in the Japan Collection of Microorganisms, Institute of Physical and Chemical Research, as strains JCM 7811, 7812, 7813, and 7814, respectively.

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