Biosci. Biotechnol. Biochem., **62** (6), 1087–1092, 1998

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Isolation of a Sulfur-oxidizing Bacterium That can Grow under Alkaline pH, from Corroded Concrete

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Received December 1, 1997

To study the early stages of concrete corrosion by bacteria, sulfur-oxidizing bacterium strain RO-1, which grows in an alkaline thiosulfate medium (pH 10.0) was isolated from corroded concreate and characterized. Strain RO-1 was a Gram negative, rod-shaped bacterium (0.5–0.6 × 0.9–1.5 μm). The mean G+C content of the DNA of strain RO-1 was 65.0 mol%. Optimum pH and temperature for growth were 8.0. and 30–37°C, respectively. When grown in thiosulfate medium with pH 10.0, growth rate of the strain was 48% of that observed at the optimum pH for growth. Strain RO-1 used sulfide, thiosulfate, and glucose, but not elemental sulfur or tetrathionate, as a sole energy source. Strain RO-1 grew under anaerobic conditions in pepton-NO_3^- medium containing sodium nitrate as an electron acceptor, and had enzyme activities that oxidized sulfide, elemental sulfur, thiosulfate, sulfite, and glucose, but not tetrathionate. The bacterium had an activity to assimilate $^{14}CO_2$ into the cells when thiosulfate was used as an energy source. These results suggest that strain RO-1 is *Thiobacillus versutus*. Strain RO-1 exuded Ca^{2+} from concrete blocks added to thiosulfate medium with pH 9.0 and the pH of the medium decreased from 9.0 to 5.5 after 22 days of cultivation. In contrast, *Thiobacillus thiooxidans* strain NB1-3 could not exude Ca^{2+} in the same thiosulfate medium, suggesting that strain RO-1, but not *T. thiooxidans* NB1-3, is involved in the early stage of concrete corrosion because concrete structures just after construction contain calcium hydroxide and have a pH of 12–13.

Key words: sulfur-oxidizing bacterium; *Thiobacillus versutus*; corroded concrete

In 1945, Parker presented evidence that concrete structures are corroded by the sulfuric acid produced by the sulfur-oxidizing bacterium *Thiobacillus concretivourus*.[1–3] Concrete in its main structure is composed of calcium oxides, hydroxides, and carbonates and these compounds can react with sulfuric acid to give calcium sulfate (gypsum).[4] This calcium loss from concrete greatly diminishes the strength of concrete structures. The bacterium isolated by Parker was later identified as *Thiobacillus thiooxidans* and thibacilli other than *T. thiooxidans*, namely *Thiobacillus neapolitanus*, *T intermedius*, and *Thiobacillus novellus*,[5–7] were also isolated from sewer systems and corroded concretes. The results accumulated up to now strongly suggest that a final rapidly corrosive stage of concrete structure is due to the activity of the obligately acidophilic chemolithoautotroph *T. thiooxidans*. However, the explanation for the preliminary stage of slow corrosion of concrete structures is not clear.[3] Since concrete structures just after construction contain calcium hydroxide and thus have a pH of 12–13, it seems that sulfur-oxidizing bacteria that can grow under alkaline pH conditions are involved in the initial stage of concrete corrosion.

We recently showed that concrete supplemented with nickel is resistant to corrosion[8] and nickel bound to the plasma membrane of *T. thiooxidans* NB1-3[9] inhibits sulfur dioxygenase and sulfite oxidase of the membrane, and as a result, inhibits cell growth.[10–12] To study the early stages of concrete corrosion and identify the biological aspects of Ni protection in concrete corrosion more precisely, we tried to isolate sulfur-oxidizing bacteria that can grow under alkaline pH conditions. Here, we show that *Thiobacillus versutus* is also present in the corroded concrete and compared with *T. thiooxidans* NB1-3 strongly exudes Ca^{2+} from carbonated concrete blocks at pH 9.5.

Materials and Methods

Microorganisms, media, and conditions of cultivation. The bacteria used in this study was *Thiobacillus thiooxidans* strain NB1-3,[9] *Thiobacillus versutus* IFO14568, *Thiobacillus novellus* IFO14993, and sulfur-oxidizing bacterium strain RO-1 isolated from corroded concrete of a sewage treatment plant in Itoman City, Okinawa Prefecture, Japan. The methods for the isolation of sulfur-oxidizing bacterium were as follows. The corroded concrete (1.0 g) was incubated under aerobic conditions at 30°C in 20 ml of thiosulfate medium (pH 9.5) containing sodium thiosulfate (0.5%), $Na_2HPO_4 \cdot 12H_2O$ (0.15%), KH_2PO_4 (1.1%), $MgSO_4 \cdot 7H_2O$ (0.01%), NH_4Cl (0.03%), and yeast extract (0.03%). When sulfur-oxidizing bacteria grew in the thiosulfate medium, samples of the culture medium were plated on gellan gum plates containing sodium thiosulfate (0.5%), $Na_2HPO_4 \cdot 12H_2O$ (0.15%), KH_2PO_4 (1.1%), $MgSO_4 \cdot 7H_2O$ (0.01%), NH_4Cl (0.03%), and yeast extract (0.03%). Colonies appearing on the plate were picked up. This process was repeated more

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than three times and the final isolates were preserved in thiosulfate medium (pH 7.5). Glucose medium (pH 7.5) contained glucose (0.2%), $Na_2HPO_4 \cdot 12H_2O$ (0.15%), KH_2PO_4 (1.1%), $MgSO_4 \cdot 7H_2O$ (0.01%), NH_4Cl (0.03%), and yeast extract (0.03%). Peptone-NO_3^- medium (pH 7.5) used for the growth of strain RO-1 under anaerobic conditions was composed of peptone (10%), NaCl (0.5%), and KNO_3 (0.1%).

Growth rate. Cells in the medium were counted with a hemacytometer (Kayagaki Irika Kogyō Co. Ltd., Tokyo) after dilution with water when necessary. Growth rate was also measured by the increase of absorbance of the culture at 660 nm.

Inorganic sulfur-oxidizing activites. The oxidation activity for inorganic sulfur compounds was estimated from the oxygen uptake rate in a Warburg manometer. A Warburg flask contained a reaction mixture (3.0 ml) in the reaction chamber and 20% potassium hydroxide (0.2 ml) in the center well. The gas phase was air and the temperature was kept at 30°C. The reaction mixture in the Warburg flask was composed of 0.1 M diethanolamine-HCl buffer (pH 9.0, 2.0 ml), washed intact cells (1.0 mg of protein), and sodium sulfide (10 μmol) for the measurement of hydrogen sulfide oxidation activity. The reaction mixtures were composed of 0.1 M MOPS buffer (pH 8.0, 2.0 ml), washed intact cells (1.0–5.0 mg of protein), and elemental sulfur (200 mg) and potassium tetrathionate (20 μmol), sodium thiosulfate (20 μmol), or sodium sulfite (20 μmol) for the measurements of elemental sulfur, tetrathionate, thiosulfate, and sulfite oxidation activities. The reaction was done by shaking the reaction mixture at 30°C. The amount of chemical oxidations of each inorganic sulfur compound was also checked with a reaction mixture containing 10-min boiled cells instead of the native cells.

Activity of $^{14}CO_2$ uptake into washed intact cells of strain RO-1. The activity of carbon dioxide (CO_2) fixation was estimated from the amount of $Na_2{}^{14}CO_3$ assimilated by the cells. The composition of the reaction mixture was as follows: 4.0 ml of 0.1 M β-alanine-SO_4^{2-} buffer, pH 3.0; washed intact cells of strain RO-1 grown in thiosulfate- or glucose-medium, 1 mg of protein; carrier Na_2CO_3, 1 μmol; $Na_2{}^{14}CO_3$, 1 μCi; and thiosulfate, 20 μmol. The total volume of the reaction mixture was 5.0 ml. The reaction mixture except sodium carbonate and thiosulfate was incubated at 30°C for 10 min. The reaction was started by adding $Na_2{}^{14}CO_3$, carrier Na_2CO_3, and thiosulfate. After 60 min of incubation at 30°C, the reaction was stopped by adding 0.5 ml of 20 mM mercuric chloride. The reaction mixture (0.55 ml) was withdrawn and then passed through a 0.45-μm membrane filter. The filter with cells was washed three times with 10 ml of water and then put into 4.0 ml of a counting sol (Scintisol EX-H). After the filter had been completely solubilized in the counting mixture, the radioactivity was measured with an Aloka LSC-635 liquid scintillation system.

Analysis of Ca^{2+} exuded from concrete blocks. Sulfur-oxidizing bacterium strain RO-1 were inoculated in pH 9.5- and pH 7.0-thiosulfate media with two pieces of carbonated concrete blocks (0.6 × 0.6 × 0.6 mm), and cultured for 24 days at 30°C. A sample of the culture medium was withdrawn, centrifuged at 12,000 × g for 15 min to discard cells, and the amount of Ca^{2+} in the supernatant was measured by atomic absorption spectroscopy with a Shimadzu AA-625-01 spectrophotometer using a air-acetylene flame. The spectral line chosen was 4227 Å. The standard solution of Ca^{2+} was prepared by dilution of the 1000 μg/ml standard calcium solution for atomic absorption spectroscopy (Ishizu Pharmaceutical Co.) with 1 N HCl.

Analysis of DNA base composition. The DNA base compositon was measured by reversed-phase high-pressure liquid chromatography after the DNA was hydrolyzed into nucleosides with enzymes.[13]

Results and Discussion

Isolation of sulfur-oxidizing bacteria that can grow under alkaline pH conditions from the corroded concrete.

Fifty-nine strains of sulfur-oxidizing bacteria were isolated from the corroded concrete of sewage treatment plants in Itoman City, Okinawa Prefecture, Japan. It has been known that *Thiobacillus novellus* and *Thiobacillus versutus* are facultative sulfur-oxidizing bacteria that can grow in thiosulfate and glucose media with the pH from neutral to slightly alkaline.[14-16] Seven representative sulfur-oxidizing bacterial strains among fifty nine isolates, *Thiobacillus novellus* IFO14993 and *Thiobacillus versutus* IFO14568, were grown in thiosulfate medium (pH 9.5) for 5 days. The levels of cell growth in the medium were estimated by the pH decrease due to the sulfuric acid production by these sulfur-oxidizing bacteria and by the increase of absorbance at 660 nm of culture medium. Strain RO-1, compared with the other eight strains, grew in the medium with a marked pH decrease (Table 1). The growth rate of strain RO-1 in the medium was faster than those of *T. novellus*

Table 1. Growth of Sulfur-oxidizing Bacteria Isolated from Corroded Concrete in Thiosulfate Medium (pH 9.5)

Strain	Growth yield[a] ($A_{660\,nm}$ / 5 d)	pH[b]
without inoculation	0.000	9.50
RO-1	0.296	6.37
RO-2	0.099	9.10
RO-3	0.112	7.35
RO-4	0.065	9.48
RO-5	0.126	9.45
RO-6	0.092	9.38
RO-7	0.125	9.35
Thiobacillus versutus IFO14568	0.142	9.31
Thiobacillus novellus IFO14993	0.087	8.92

[a] Growth rate was measured by the increase of absorbance at 660 nm of the culture.
[b] The pH of the culture was measured after 5 days of cutivation.

and *T. versutus*. We selected strain RO-1 as the representative of sulfur-oxidizing bacterium isolated from corroded concrete that grows well in alkaline thiosulfate medium.

Growth of sulfur-oxidizing bacterium strain RO-1 in inorganic sulfur media

Figure 1 shows a micrograph of strain RO-1. The strain was a Gram negative, non-spore-forming, and rod-shaped bacterium (0.5–0.6 × 0.9–1.5 μm). The mean G+C content of the DNA of this strain was 65.0 mol%. Taylor and Hoare reported that the mean G+C contents of the DNA of *T. novellus* and *T. versutus* (*Thiobacillus* A2) grown in thiosulfate medium was 68 and 65 mol%, respectively.[15] The growth rate of strain RO-1 was similar to *T. novellus* IFO14993 and *T. versutus* IFO14568 when cultivated in the medium containing thiosulfate or glucose as an energy source (Table 2), indicating that strain RO-1 is a facultative chemolithoautotroph. Strain RO-1, *T. novellus* IFO14993, and *T. versutus* IFO14568 consumed thiosulfate in the medium 14.8, 8.0, and 8.0 mM per 7 days, respectively. Only *T. novellus* IFO14993 among the three sulfur-oxidizing bacteria could use tetrathionate as an energy source for growth. The amount of tetrathionate in the medium consumed by strain RO-1, *T. novellus* IFO14993, and *T. versutus* IFO14568 were 0.0, 0.0, and 2.2 mM per 7 days, respectively. Taylor and Hoare reported that *T. versutus* does not use tetrathionate as an energy source.[15] Among three sulfur-oxidizing bacteria, only *T. novellus* IFO14993 used elemental sulfur as an energy source.

Enzyme activities that oxidize reduced inorganic sulfur compounds and glucose were measured with washed intact cells of strain RO-1, *T. novellus* IFO14993, and *T. versutus* IFO14568 grown in thiosulfate and glucose media (Table 3). When grown in thiosulfate medium, these three sulfur-oxidizing bacteria had enzyme activities that oxidize sodium sulfide, sodium thiosulfate, sodium sulfite, and glucose. Strain RO-1 and *T. versutus* IFO14568, unlike *T. novellus* IFO14993, could not oxidize tetrathionate. These results support the idea that *T. novellus* IFO14993, but not Strain RO-1 and *T. versutus* IFO14568, grew in tetrathionate medium. When grown in glucose medium, thiosulfate-oxidizing activities of these three sulfur-oxidizing bacteria were markedly repressed.

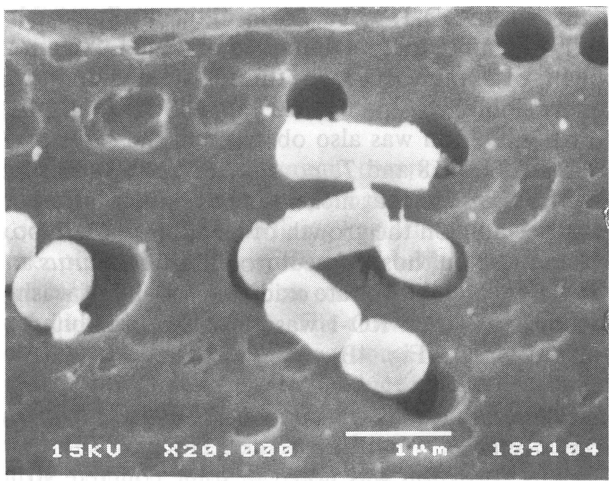

Fig. 1. Electron Micrograph of Strain RO-1. Bar represents 1.0 μm.

Table 2. Growth of Stram RO-1, *T. versutus*, and *T. novellus* on the Medium Supplemented with Inorganic Sulfur or Glucose as an Energy Source

Strain	Energy source			
	Elemental sulfur	thiosulfate	tetrathionate	Glucose
Strain RO-1 $A_{660\,nm}$/7 d[a]	0.000	0.105	0.029	0.497
T. versutus IFO14568 $A_{660\,nm}$/7 d	0.004	0.077	0.030	0.896
T. novellus IFO14568 $A_{660\,nm}$/7 d	0.027	0.055	0.144	1.060

[a] Growth rate was measured by the increase of absorbance of the medium at 660 nm.

Table 3. Enzyme Activities that Oxidize Reduced Inorganic Sulfur Compounds and Glucose

Substrate	Oxidation activity (μl/mg/min)		
	Strain RO-1	*T. versutus* IFO14568	*T. novellus* IFO14993
Intact cells grown on thiosulfate medium			
Sodium sulfide	2.52	2.29	1.53
Elemental sulfur	0.60	0.35	0.00
Sodium thiosulfate	2.33	2.25	4.02
Potassium tetrathionate	0.00	0.00	2.81
Sodium sulfite	0.56	0.07	3.73
Glucose	0.84	0.12	0.28
Intact cells grown on glucose medium			
Sodium thiosulfate	0.17	0.18	0.10
Glucose	0.84	0.76	0.38

Activity of carbon dioxide fixation

$^{14}CO_2$ fixation activity of strain RO-1 was studied with washed intact cells grown in thiosulfate and glucose media (pH 8.0). Thiosulfate grown-cells assimilated $^{14}CO_2$ when thiosulfate was used as an energy source (Fig. 2). The specific activity of $^{14}CO_2$ fixation was 291 nmol/mg/60 min. However, when glucose was used as an energy source, the activity markedly decreaased (11 nmol/mg/60 min). The glucose grown strain RO-1 did not have $^{14}CO_2$ fixation activity, indicating that strain RO-1 grews autotrophically in thiosulfate medium with CO_2 as a carbon source and heterotrophically in glucose medium with glucose as a carbon source.

Optimum pH and temperature for growth

Strain RO-1, *T. versutus* IFO14568, and *T. novellus* IFO14993 had optimum pHs for growth at 7.0, 7.0, and 8.0, respectively (Fig. 3A). It is interesting that strain RO-1 grew better in the medium with alkaline pH than *T. versutus* IFO14568 and *T. novellus* IFO14993. The

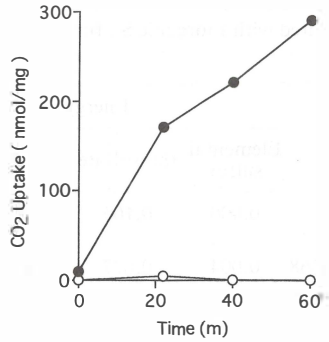

Fig. 2. $^{14}CO_2$ Uptake Activity of Sulfur-oxidizing Bacterium Strain RO-1 Isolated from Corroded Concrete.

$^{14}CO_2$ uptake activities were measured in the reaction mixture with (○) or without (●) 20 mM $HgCl_2$.

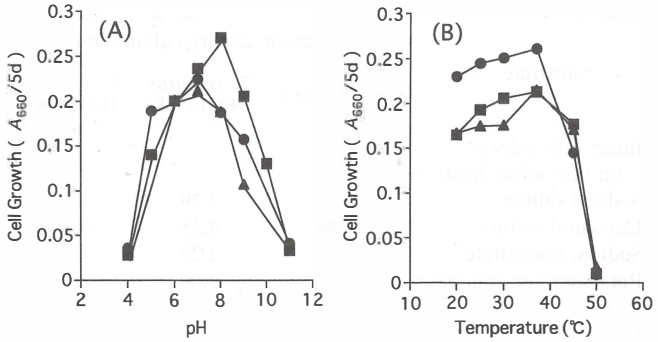

Fig. 3. Effects of pH (A) and Temperature (B) on the Growth of Sulfur-oxidizing Bacterium Strain RO-1, *T. versutus*, and *T. novellus* in Thiosulfate Medium.

Sulfur-oxidizing bacterium strain RO-1 (■), *T. versutus* IFO14568 (●), and *T. novellus* IFO14993 (▲) were statically grown in 20 ml of thiosulfate medium for 5 days.

Table 4. Characteristics of Sulfur-oxidizing Bacterium Strain RO-1 Isolated from Corroded Concrete

Characteristics	Strain RO-1	*T. versutus* IFO14568	*T. novellus* IFO14993
Gram stain	negative	negative	negative
Morphology	rod-shaped	rod-shaped	rod-shaped
Motility	motile	motile	non-motile
G+C content (mol%)	65	65	68
Optimum pH for growth	8	7	7
Optimum temperature for growth (°C)	30–37	30–35	25–30
Energy source	sulfide	sulfide	sulfide
	thiosulfate	thiosulfate	thiosulfate
			tetrathionate
	glucose	glucose	glucose
Oxidizing activity for			
Sodium sulfide	+	+	+
Elemental sulfur	+	+	−
Sodium thiosulfate	+	+	+
Potassium tetrathionate	−	−	+
Sodium sulfite	+	+	+
Glucose	+	+	+
Ability to grow by nitrate respiration	+	+	−

growth rates of strain RO-1 in thiosulfate medium with pH 9.0 and 10.0 were 75 and 48% of the optimum growth of this bacterium. The three sulfur-oxidizing bacteria did not grow in thiosulfate medium with pHs above 11.0. Strain RO-1, like *T. versutus* IFO14568, had an optimum temperature for growth at 30–37°C (Fig. 3B).

The properties of strain RO-1 are summarlized in Table 4. The most remarkable points found in strain RO-1 and *T. versultus* IFO14568 were the cells' ability to grow under anaerobic conditions in the medium containing sodium nitrate as an electron acceptor and its inability to oxidize tetrathionate as an energy source. Strain RO-1 can grow in media with neutral and slightly alkaline pHs not only autotrophically with CO_2 as a carbon source and thiosulfate as an energy source but also heterotrophically with glucose as carbon and energy sources. It has been known that the facultative sulfur-oxidizing bacterium *Thiobacillus intermedium* grows in media with pHs from 3.0 to 7.0, but this bacterium cannot grow under anaerobic conditions.[17] Another facultative sulfur-oxidizing bacterium, *Thiobacillus acidophilus*, grows in elemental-sulfur media with pHs from 2.0 to 4.0, but the bacterium also cannot grow under anaerobic conditions.[18] *Thiobacillus denitrificans*[19] and *Thiobacillus neapolitanus* grow in media with pHs from 5.0 to 8.0, but they are obligate chemolithoautotrophs and cannot use glucose as energy and carbon sources.[14] The value of the mean G+C content of the DNA of strain RO-1 corresponded with that of *T. versutus*. The results obtained in this report suggest that strain RO-1 is *T. versutus*.

Effects of nickel sulfate on the growth of strain RO-1

Nickel added to concrete protects the concrete from corrosion.[8] The causes of protection by nickel from corrosion were studied with the acidophilic, chemolithoautotrophic, sulfur-oxidizing bacterium *Thiobacillus thiooxidans* NB1-3 isolated from corroded concrete.[9,10] We showed that nickel binds to the plasma membrane of this strain and inhibits sulfur dioxygenase and sulfite oxidase of the membrane, and as a result, inhibits cell growth.[10] Therefore, it is very interesting to study whether strain RO-1, which grows in the medium with a slightly alkaline pH, is inhibited by $NiSO_4$ or not. Growth of strain RO-1 in thiosulfate medium was strongly inhibited by 0.3 mM of $NiSO_4$ (Fig. 4A). Growth inhibition was also observed in the cases of *T. versutus* IFO14568 and *T. novellus* IFO14993 (data not shown), suggesting that nickel ion has similar inhibitory effects not only on the growth of acidophilic *T. thiooxidans* but also on those of neutorophilic *T. versutus* and *T. novellus*. The thiosulfate oxidizing activity of washed intact cells of strain RO-1 was completely inhibited by 10 mM of $NiSO_4$ (Fig. 4B).

Exudation of Ca^{2+} from concrete blocks by sulfur-oxidizing bacteria

Serious corrosion was noted in some concrete structures used for sewage treatment.[1-6] In the process of concrete corrosion, sulfate-reducing bacteria produce

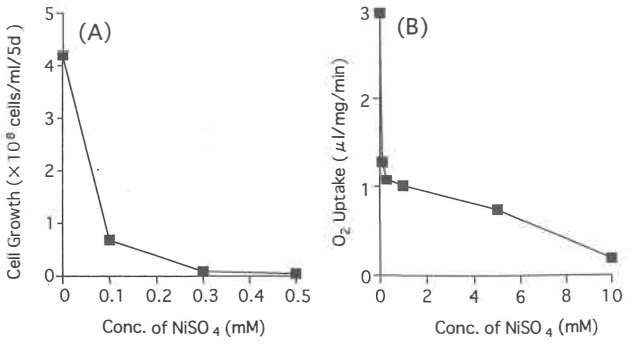

Fig. 4. Effects of $NiSO_4$ on the Growth in Thiosulfate Medium (A) and on Thiosulfate Oxidation Activity (B) of Sulfur-oxidizing Bacterium Strain RO-1.

(A) Sulfur oxidizing bacterium strain RO-1 was grown in thiosulfate medium (pH 8.0) with or without $NiSO_4$ for 5 days and the levels of growth were measured by counting cells with a hemacytometer. (B) Thiosulfate oxidizing activity was measured from the oxygen uptake caused by the oxidation of thiosulfate in a Warburg manometer.

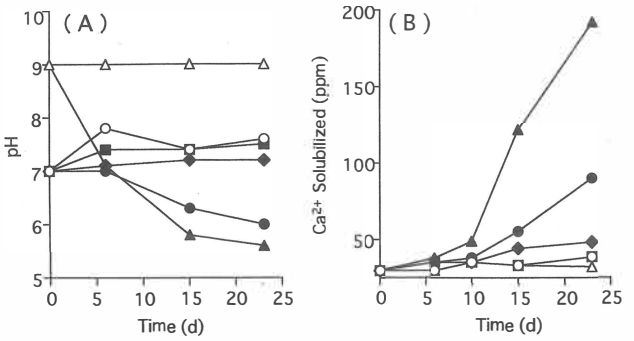

Fig. 5. Solubilization of Ca^{2+} from Concrete Blocks by Sulfur-oxidizing Bacterium Strain RO-1 and *Thiobacillus thiooxidans* Strain NB1-3.

Sulfur-oxidizing bacterium strain RO-1 and *T. thiooxidans* strain NB1-3 were grown on thiosulfate media (pH 9.0 and pH 7.0) containing three pieces of carbonated concrete blocks (6×6×6 mm). (A) pH change. (B) Solubilization of calcium ion from concrete blocks. Ca^{2+} solubilized from the concrete blocks were measured by atomic absorption spectrophotometer. Symbols: △, without inoculation (pH 9.0); ○, without inoculation (pH 7.0); ▲, Strain RO-1 (pH 9.0); ●, Strain RO-1 (pH 7.0); ◆, *T. thiooxidans* strain NB1-3 (pH 7.0); ■, Strain RO-1 (pH 7.0) in the presence of 1 mM of $NiSO_4$.

hydrogen sulfide from organic compounds and sulfate ion in the sewage and the hydrogen sulfide thus formed is oxidized by sulfur-oxidizing bacteria on the surface of sewer pipes to produce sulfuric acid. The concrete structure just after construction contains calcium hydroxide and thus has a pH 12–13. The calcium hydroxide in the concrete structure, then, is carbonated with carbon dioxide in the atmosphere to produce calcium carbonate, decreasing the pH of the concrete structure from 12–13 to 8.5–10. This pH decrease is known as neutralization of the concrete structure and concrete corrosion is increased after this neutralization.[20] Sulfuric acid produced by sulfur-oxidizing bacteria not only prompts the neutralization of concrete but also exudes Ca^{2+} from the concrete structure as calcium salfate, causing a rapid decomposition of the concrete.[1-3] Thus, it seems reasonable that the level of concrete corrosion caused by sulfur-oxidizing bacteria can be estimated by the concentration of Ca^{2+} exuded from concrete blocks. Strain RO-1 had the ability to exude Ca^{2+} from carbonated concrete blocks in the thiosulfate medium and the pHs of the media decreased from 9.0 to 5.5 after 22 days of cultivation (Fig. 5). Exudation of Ca^{2+} by strain RO-1 in the pH 9.0 medium was faster than the pH 7.0 medium, suggesting that strain RO-1 exudes Ca^{2+} from concrete blocks more rapidly undrer alkaline pH than at neutral pH. *Thiobacillus thiooxidans* strain NB1-3,[9] acidophilic sulfur-oxidizing bacterium isolated from corroded concrete, could not exude Ca^{2+} from concrete blocks in the thiosulfate medium with pH 7.0 and pH 9.0, suggesting that strain RO-1, but not *T. thiooxidans* NB1-3, is involved in the early stage of concrete corrosion. The activity of strain RO-1 to exude Ca^{2+} from concrete blocks was markedly inhibited by 0.1 mM of $NiSO_4$.

A facultative sulfur-oxidizing bacterium, *T. versutus* strain RO-1, was first isolated from corroded concrete of a sewage treatment plant. The results that strain RO-1, but not *T. thiooxidans* NB1-3, exudes Ca^{2+} from carbonated concrete blocks strongly suggests that the former is more important in early stages of concrete corrosion than the latter. Optimum pH for growth of strain RO-1 was one pH unit higher than that of *T. versutus* IFO14568. However, strain RO-1 could not grow in thiosulfate medium with the pH above 11. Thus, strain RO-1 probably attack concrete structures after they are carbonated and neutralized by carbon dioxide in the atmosphere to pH 8.5–10. It is interesting that not only *T. thiooxidans* NB1-3 but also strain RO-1 was strongly inhibited by Ni^{2+}. In *T. thiooxidans* NB1-3, the Ni^{2+} strongly binds to the plasma membrane and inhibits sulfur dioxygenase and sulfite oxidase of the membrane, and as a result, inhibits cell growth.[9-12] To study the protection of concrete from corrosion by nickel more precisely, the mechanism of growth inhibition of strain RO-1 by Ni^{2+} is now under examination.

References

1) C. D. Parker, *Aust. J. Exp. Biol. Med. Sci.*, **23**, 81–90 (1945).
2) C. D. Parker, *Aust. J. Exp. Biol. Med. Sci.*, **23**, 91–98 (1945).
3) C. D. Parker, *Nature*, **159**, 439–440 (1947).
4) W. Sand, *Appl. Environ. Microbiol.*, **53**, 1645–1648 (1987).
5) K. Milde, W. Sand, W. Wolff, and E. Bock, *J. Gen. Microbiol.*, **129**, 1327–1333 (1983).
6) W. Sand and E. Bock, *Environ. Tech. Lett.*, **5**, 517–528 (1984).
7) N. Yoshida, T. Morinaga, and Y. Murooka, *J. Ferment. Bioeng.*, **76**, 400–402 (1993).
8) T. Maeda and A. Negishi, in "Fracture and Damage of Concrete and Rock-FDCR-2," ed. by H. P. Rossmanith, © E & FN Spon, 1993.
9) T. Maeda, A. Negishi, Y. Nogami, and T. Sugio, *Biosci. Biotech. Biochem.*, **60**, 626–629 (1996).
10) Y. Nogami, T. Maeda, A. Negishi, and T. Sugio, *Biosci. Biotech. Biochem.*, **61**, 1373–1375 (1997).
11) T. Sugio and T. Maeda, *Bio Industry* (in Japanese), **13**, 13–20 (1996).
12) T. Maeda, *Building Maintenance & Management* (in Japanese),

105, 37–42 (1997).
13) J. Tamaoka and K. Komagata, *FEMS Microbiol. Lett.*, **25**, 125–128 (1984).
14) M. Santer, J. Boyer, and U. Santer, *J. Bacteriol.*, **78**, 197–202 (1959).
15) B. F. Taylor and D. S. Hoare, *J. Bacteriol.*, **100**, 487–497 (1969).
16) J. G. Kuenen and O. H. Tuovinen, in "The Prokaryotes," ed. by M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel, Springer-Verlag, Berlin Heidelberg New York, 1981, pp. 1023–1036.
17) J. London, *Arch. Mikrobiol.*, **40**, 329–337 (1963).
18) R. Guay and M. Silver, *Can. J. Microbiol.*, **21**, 281–288 (1974).
19) B. F. Taylor, D. S. Hoare, and S. L. Hoare, *Arch. Mikrobiol.*, **78**, 193–204 (1974).
20) T. Maeda, in "Chuseika," (Neutralization) (in Japanese), ed. by I. Izumi, T. Kita, T. Maeda, Gihodou Press, Tokyo, 1986, pp. 1–4.