The Journal of Microbiology, December 1999, p.206-212
Vol. 37, No. 4

Isolation and Identification of an Anaerobic Dissimilatory Fe(III)-Reducing Bacterium, *Shewanella putrefaciens* IR-1

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(Received November 18, 1999 / Accepted December 7, 1999)

In order to isolate a Fe(III)-reducer from the natural environment, soil samples were collected from various paddy fields and enriched with ferric citrate as a source of Fe(III) under anaerobic condition. Since the enrichment culture was serially performed, the Fe(III)-reduction activity increased. The final enriched culture that showed the highest Fe(III)-reduction activity was serially diluted and cultivated on an agar plate containing lactate and ferric citrate in an anaerobic glove box. A Gram negative, motile, rod-shaped and facultative anaerobic Fe(III)-reducer was isolated based on its highest Fe(III)-reduction activity. Bacterial growth was coupled with oxidation of lactate to Fe(III)-reduction, but the isolate fermented pyruvate without Fe(III). The isolate reduced an insoluble ferric iron (FeOOH) as well as a soluble ferric iron (ferric citrate). Using the BBL crystal enteric/non-fermentor identification kit and 16S rDNA sequence analysis, the isolate was identified as *Shewanella putrefaciens* IR-1.

Key words: *Shewanella putrefaciens*, Fe(III)-reducer, metal reduction

Metal oxy-anions including Fe(III) are used as electron acceptors by anaerobic bacteria (19). The turnover of organic matter coupled to a microbial dissimilatory reduction of Fe(III) has been described as one of the most important geochemical reactions taking place in anaerobic ecosystems. This Fe(III)-reduction generally occurred in aquatic and marine sediments, and submerged soils (5, 6). In sedimentary environments, the oxidation of organic matter coupled to the reduction of Fe(III) requires the cooperative activity of several bacteria (19). For example, a variety of fermentative bacteria that can metabolize sugars or amino acids produce acids, alcohols, and H_2 as their primary fermentation products. These products are oxidized further to CO_2 with production of secondary fermentation products, such as H_2 or formate. These fermentation products are oxidized with the coupling of Fe(III)-reduction by dissimilatory Fe(III)-reducers. However, these Fe(III)-reducers must compete with other anaerobic bacteria, such as sulfate reducers, methanogens, and acetogens for these electron donors. Fe(III)-reducers would be more thermodynamically energetic than other anaerobic respiratory bacteria (18, 19). Therefore, Fe(III)-reducer outcompete these anaerobic respiratory bacteria in the presence of Fe(III) (18, 19). On the other hand, fermentative Fe(III)-reducers that metabolize sugars with the coupling of Fe(III)-reduction have been described by several workers (11,18). However, in all the cases examined, this Fe(III)-reduction has so far been reported as a side reaction in the metabolism of these organisms. This indicates that the primary products of the metabolism of these fermentative Fe(III)-reducers are very similar to general fermentative bacteria.

Based on their carbon metabolisms, dissimilatory Fe(III)-reducers can be divided into two groups. First group could be organisms that completely oxidize multi-carbon compounds to CO_2, and the second group could be organisms that incompletely oxidize multi-carbon compounds and produce acetate. The complete oxidizer includes *Geobacter* (4, 20), *Desulfuromonas* (9), and *Desulfuromusa* (10) species, while the incomplete oxidizer includes *Shewanella* (22), *Pelobacter* (23), and *Geospirillum barnesii* (16).

Shewanella putrefaciens was originally classified as a member of the *Pseudomonas* group IV (29). Later, this strain was transferred to the genus of *Alteromonas* due to its G+C content of 44 mol %, as opposed

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to the 58 to 65 mol % for the genus *Pseudomonas* (3). MacDonell and Colwell (25) reclassified this organism in a new genus, *Shewanella*, using a similarity in the 5S rRNA sequence.

S. putrefaciens does not produce a soluble and extracellular component such as enzymes for Fe(III)-reduction. However, this bacterium is known to reduce water-insoluble Fe(III) (2). Fe(III), used as an electron acceptor by the Fe(III)-reducing bacteria such as *Shewanella putrefaciens*, is essentially insoluble in water at neutral pH. Therefore, physical contact between the bacterial cells and Fe(III) minerals is necessary for the reduction of the water insoluble electron acceptor (15). The electron transfer phenomena between the bacterium and the insoluble electron acceptor could be conveniently estimated using cyclic voltammetry (13), which showed that intact cells of anaerobically grown *S. putrefaciens* are electrochemically active (13, 14). A mediator-less microbial fuel cell can be constructed to generate electricity from substrates that may serve as electron donors for electrochemically active bacteria (14). This will lead to the development of not only a novel wastewater treatment process but also a method to generate electricity using the organic contaminants as fuel. The microbial fuel cell using electrochemically active bacteria can also be used as a simple biosensor (i.e. without transducer or electrochemical mediator) to measure the concentration of electron donors. Excess reducing-equivalent can also be removed electrochemically from a bioremediation process using Fe(III)-reducers which are able to metabolize a wide range of organic contaminants (24).

In this study, we describe cultural technique used to isolate Fe(III)-reducers from natural environments, and show the physiological characteristics of a mesophilic Fe(III)-reducer that was isolated from a rice-paddy in local areas.

Materials and Methods

Enrichment and isolation of Fe(III)-reducers

A variety of samples from the environment were taken. Typically, they involved samples from waters, sediments and soils that were transferred to anaerobic vials or taken back to the laboratory prior to handling according to the modified Hungate technique (17). The liquid samples were transferred to nitrogen filled pressure tubes (2.5 × 15 cm, Bellco Glass Inc.). The solid samples were moved to the laboratory without oxygen contact, and 1 g of solid sample was suspended in 10 ml of anaerobic saline solution. One ml of each sample (liquid and suspended soil samples) was transferred to pressure tubes containing 10 ml of carbonate buffered basal medium (CBBM) (28) with lactate and FeOOH. The tube was incubated at 30°C for 7 days in the dark condition. The enrichment procedure was repeated 3 times. Enriched cultures were serially diluted and plated on solid phosphate buffered basal medium (PBBM) (28) containing sodium lactate and ferric citrate in an anaerobic glove box (Coy Lab. Products, Inc.). Colonies which appeared on the plate were isolated to test their Fe(III)-reduction activities.

Bacterial strains, media and culture techniques

Strain IR-1 was selected and used throughout the study for its highest Fe(III)-reduction activity. *Shewanella putrefaciens* MR-1 (27) was used as a type strain. CBBM was used to cultivate and maintain the culture. One L of basal medium without a carbonate source was boiled to remove dissolved oxygen, and cooled under an oxygen-free gas mixture (N_2/CO_2, 8/2). Twenty five ml of Na_2CO_3 solution (16% (w/v)) was added to the medium before pH adjustment, and then pH was adjusted to 7.4~7.6. The medium was anaerobically dispensed into pressure tubes or serum vials (160 ml, Wheaton, Milliville, NJ, USA) which were sealed with butyl rubber stoppers (Bello Glass Inc.). Sodium lactate was used as an electron donor at a final concentration of 30 mM. Amorphous ferric oxyhydroxide (FeOOH, 0.1 g/10 ml) was prepared according to LcLaughlins method (26), and supplemented to the medium as an electron acceptor. Ferric citrate (5 g/L, $FeC_6H_5O_7$, Sigma Co.) was used as a soluble Fe(III) source during cultivation of Fe(III)-reducers on an agar plate. In this culture, PBBM was used as a basal medium instead of CBBM. In order to determine the optimum temperature for the growth of the isolate, the organism was inoculated to serum vials (anaerobic condition) or baffle flasks (aerobic condition) containing LB broth or PBBM with/without Fe(III) sources. These vessels were kept in 25, 30, 37, and 55°C water baths (anaerobic condition) or shakers (aerobic condition, 150 rpm). In a similar manner, optimum pH for the growth of the isolate was also determined. The pH of media was adjusted by acetate buffer (10 mM, for pH 5.5), phosphate buffer (10 mM, for pH 6.8), or glycine-NaOH buffer (10 mM, for pH 8.0). One ml of disodium phosphate solution (1M) was added to acetate or glycine-NaOH buffered media to supply phosphorous (7). Cultural characteristics of *S. putrefaciens* IR-1 was investigated at 37°C with PBBM (100 ml) containing sodium lactate (15 mM) or sodium pyruvate (10 mM) with/without Fe(III) sources.

Analyses

Fe(III) reduction activity was determined using a previous method (21). A half ml of culture was aseptically sampled with a syringe and mixed with 1 ml of

HCl solution (0.5 N). This mixture was reacted for 15 min at room temperature, and then centrifuged (10,000× g, HM 150IV; Hanil Co.) for 5 min. The mixture of supernatant (0.1 ml) and ferrozine solution (1 ml, 1 g/L in 50 mM HEPES buffer [pH 7.0]) was reacted for 15 min before measurement of optical density at 562 nm using a spectrophotometer (Uvi-Dec 610, Jasco). The fresh medium was used as a control sample instead of culture. Ferrous ethylenediammonium sulfate tetrahydrate ($C_2H_{10}N_2O_4SFeSO_44H_2O$, Fluka Chemie AG) was used to make a standard Fe(II) solution. The cellular protein concentration was measure by the Biuret method (12) with bovine serum albumin as a standard. Fatty acids were analyzed by HPLC (Young-Lin) equipped with an Aminex HPX-87H column (Bio-Rad) at 210 nm (8). The mobile phase used was 0.005N sulfuric acid at a flow rate of 0.6 ml/min. Carbon dioxide was measured by a thermal conductivity detector of a GC (Varian 3400) as previously described (7).

Determination of morphological characteristics

Lactate-grown cells were used for Gram staining. The stained cells were observed by a light microscope (Jenalumar, Carl Zeiss). Using a scanning electron microscope (SEM), similar cells were observed. Prior to the observation using SEM (S-4200 FE-SEM, Hitachi), cells were treated as previously described (7). The motility test was determined by diffusion of colony on PBBM/ferric citrate agar plates 0.5%(w/v) of agar for 2 days at 30°C.

Determination of physiological and biochemical characteristics

Indole-Nitrate dehydrated medium (25 g/L, BBL, Becton Dickinson Microbiology Systems) was used to test indole production. Oxidase and catalase tests were performed with commercial kits (Dryslide™ Oxidase Kit for oxidase, Spot test™ Catalase test for catalase, Difco Laboratories). The biochemical characteristics of the isolate were determined using a BBL crystal enteric/nonfermenter ID kit. The isolate was incubated on CBBM with lactate/FeOOH for 4 days. One ml of culture was diluted with 9 ml of saline solution, and 0.1 ml of this diluted solution was incubated on the PBBM/ferric citrate agar plate for 7 days. A single colony which was collected was used in the BBL crystal enteric/nonfermenter ID kit method. The results obtained from the kit were compared with the database which was supplied by BBL.

16S rDNA sequencing

Chromosomal DNA was isolated as described previously (30), and 16S rDNA was sequenced using a MicroSeqTM 16S rRNA Gene Kit (Perkin Elmer, Applied Biosystems). The sequence was aligned with corresponding representative *Shewanella* strains available from public databases by BLAST Search (http://www/ncbi.nlm.nih.gov/) (1). The 16S rDNA sequence determined in this study has been deposited in the GenBank under accession number AF170300.

Results

Enrichment and isolation

Samples were collected from various anaerobic habitats including soils (15 sites), sediments (18 sites), and rice paddies (13 sites). As mentioned in the Materials and Methods section, whole samples were aseptically handled under anaerobic condition. During the first enrichment cultures, pH did not change and Fe(III)-reduction activities (over 1 mM) were observed in most samples. However, repeated transfers yielded different Fe(III)-reduction activities among the enriched cultures. One of the enriched cultures, initially collected from paddy fields, showed the highest Fe(III)-reduction activity. This culture was serially diluted in an anaerobic saline solution, and inoculated on a PBBM/citrate agar plate in an anaerobic glove box. During the cultivation under anaerobic condition, over 10^8 CFUs/ml were measured for 7 days. Colonies were isolated, and further tested for their Fe(III)-reduction activities. From over 120 isolates, isolate IR-1 was selected based on its highest Fe(III)-reduction activity.

Morphological and physiological characteristics

Observation of lactate/FeOOH-grown cells of the isolate IR-1 by SEM showed that isolate IR-1 is a rod-shaped organ-

Fig. 1. Scanning electron micrograph of the isolate IR-1.

Table 1. The comparisons of morphological, physiological and biochemical characteristics between the isolate IR-1 and *Shewanella putrefaciens* MR-1

Characteristics and substrate utility	Isolate IR-1	*Shewanell a-putrefa-ciens* MR-1
Morphological characteristics		
Gram staining	-[a]	-
Cell morphology	rod	rod
Cell diameter(μm)	0.5~1.75	n.d.[c]
Motility	+[b]	+
Colony color	pale red	pale red
Physiological characteristics		
Facultative anaerobe	+	+
Fe(III)-reduction activity	+	+
Oxidase	+	+
Catalase	+	+
Indole test	-	-
Lactate metabolite with Fe(III)	acetate, pyruvate	acetate
Biochemical characteristics		
ARA(Arabinose)	+	-
MNS(Mannose)	-	-
SUC(Sucrose)	+	-
MEL(Melibiose)	-	-
SOR(Sorbitol)	-	-
MNT(Mannitol)	-	-
ADO(Adonitol)	-	-
GAL(Galactose)	-	-
INO(Inositol)	-	-
RHA(Rhamnose)	-	-
PHO(p-nitrophenyl phosphate)	+	+
BGL(p-nitrophenyl α-β-glucoside)	+	+
NPG(p-nitrophenyl β-galactoside)	-	-
PRO(Nitrophenyl proline)	+	+
BPH(p-nitrophenyl bis-phosphate)	+	+
BXY(p-nitrophenyl xyloside)	-	-
AAR(p-nirtophenyl α-arabinoside)	-	-
PHC(p-nitrophenyl phosphorylcholine)	+	+
GLR(p-nitrophenyl-β-glucuronide)	-	-
NAG(p-nitrophenyl-N-acetyl glucosaminide)	+	+
GGL(γ-L-glutamyl p-nitroanidine)	+	+
ESC(Esculin)	-	-
PHE(p-nitro-DL-phenylalanine)	-	-
URE(Urea)	+	+
GLY(Glycine)	+	+
CIT(Citrate)	+	+
MLO(Malonate)	-	-
TTC(tetrazolium)	-	-
ARG(Arginine)	+	+
LYS(Lysine)	-	-

[a]+: positive reaction, [b]–: negative reaction, [c]n.d.: data not available

ism, and the size was approximately 0.5 by 1.75 μm (Fig. 1). The cells were Gram negative and motile (Table 1). The isolate IR-1 showed catalase and oxidase activities, but it does not produce indole (Table 1). These data were compared with the type strain, *Shewanella putrefaciens* MR-1.

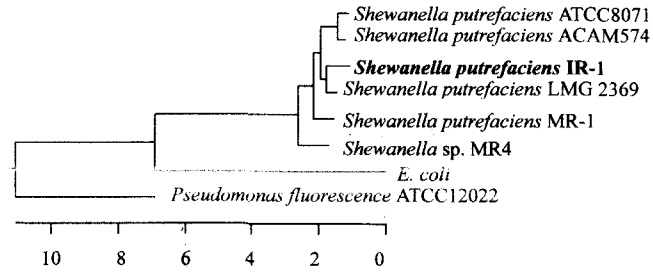

Fig. 2. Phylogenic relationships based on the results of a maximum likelihood analysis of 16S rDNA sequences.

Biochemical and phylogenetical characteristics

Other biochemical characteristics (Table 1) were tested using the BBL identification kit. The result from a computer card book search (supplied by BBL) showed that isolate IR-1 was closely related to *Shewanella putrefaciens* (ID No. 7243310212) with 99.99% confidence. However, isolate IR-1 can use arabinose and sucrose while the type strain MR-1 does not (Table 1). DNA extracted from the isolate IR-1 was used to amplify and sequence 16s rDNA (Fig. 2). The sequence was compared with 16S rDNA sequences in the BLAST search. The comparison showed that the sequence of the isolate IR-1 was closely related to the sequences of both *Shewanella putrefaciens* LMG2369 (accession No. AJ000213) and MR-1 (accession No. AF005251) with 98% homology. Less homology was found with other species of the genus *Shewanella*, 96% with *S. frigidimarina* (accession No. U85907), 95% with *S.alga* (accession No. U91545), 94% with *S. benthica* (accession No. AB003189), and 94% with *S. gelidimarina* (accession No. U85907), respectively. This result confirms that the isolate IR-1 is a member of *S. putrefaciens*. Based on these experimental results, the isolate IR-1 was identified as *Shewanella putrefaciens*.

Cultural characteristics

Improved growth was obtained in complex medium (LB broth) than in defined medium (PBBM) under anaerobic as well as aerobic conditions, and this organism was determined as a mesophilic and neutrophilic bacterium (data not shown). Table 2 shows the cultural characteristics of *S. putrefaciens* IR-1 on PBBM under optimum condition. *S. putrefaciens* IR-1 fermented pyruvate with the production of lactate, formate, acetate, and CO_2, while lactate was not fermented. Lactate was incompletely oxidized to acetate under anaerobic condition in the presence of FeOOH as an electron acceptor. On the other hand, pyruvate and lactate were completely oxidized to CO_2 under aerobic condition. These results indicate that *S. putrefaciens* IR-1 is a facultative anaerobic dissimilatory Fe(III)-reducer and Fe(III)-reduction could be coupled

Table 2. Cultural characteristics of *Shewanella putrefaciens* IR-1

Culture condition	Electron acceptor	Substrate consumed (μmol/vial)		Cell protein (mg/vial)	Fe(III) reduced (μmol/vial)	Products formed (μmol/vial)				
		Lactate	Pyruvate			Pyruvate	Lactate	Formate	Acetate	CO_2[c]
Anaerobic	FeOOH	113.8 ± 9.5	-[a]	0.87 ± 0.10	3.8 ± 0.5	-	-	-	60.0 ± 3.5	6.12 ± 0.7
	Ferric citrate	345.9 ± 15.7	-	2.03 ± 0.21	192.3 ± 15.0	18.7 ± 0.25	-	-	231.7 ± 2.4	30.8 ± 3.4
	-	71.0 ± 0.5	-	0.21 ± 0.05	-	-	-	-	72.2 ± 5.3	3.6 ± 0.4
	FeOOH	-	978.1 ± 5.3[b]	2.76 ± 0.10	26.6 ± 5.0	-	231.1 ± 3.0	396.7 ± 6.5	673.5 ± 28	79.3 ± 3.3
	Ferric citrate	-	796.6 ± 41.3[b]	1.80 ± 0.25	231.9 ± 49.1	-	108.5 ± 4.6	288.7 ± 17.7	806.4 ± 4.4	116.9 ± 7.8
	-	-	855.4 ± 20.3[b]	1.83 ± 0.38	-	-	245.9 ± 12.5	406.3 ± 8.0	677.1 ± 25.0	68.5 ± 3.4
	FeOOH	-	-	0.36 ± 0.07	0.8 ± 0.05	-			52.7 ± 3.9	4.6 ± 0.3
	Ferric citrate	-	-	1.17 ± 0.10	6.3 ± 7.5	-			91.3 ± 12.0	16.6 ± 2.6
Aerobic	O_2	1,052 ± 21.0[b]		29.38 ± 2.5		-	-	-	-	-
	O_2	-	850 ± 12.5[b]	30.84 ± 3.0		-	-	-	-	-

The cultures were performed using vials (anaerobic condition) or flasks (aerobic condition) containing PBBM (100 ml) with/without electron donors and acceptors. The data were obtained from duplicate cultures after 4 days.

[a]not available or not detected

[b]substrate was completely consumed

[c]was measured in the gas phase.

with oxidation of lactate as well as pyruvate. When soluble ferric citrate was used as an electron acceptor, even though improved growth was observed when compared to the culture in the presence of insoluble ferric iron (FeOOH), Fe(III) reduction was detected in the culture without the electron donor (Table 2). This result indicated that soluble ferric ion could be chemically reduced. In addition, the culture without an electron donor showed that bacterial growth could occur in the presence of ferric citrate. These results indicate that a part of ferric citrate could be used as an electron donor for the growth of *S. putrefaciens* IR-1. Because of its slow growth, *S. putrefaciens* IR-1 was further cultivated on PBBM/FeOOH to determine its maximum cell growth and Fe(III)-reduction activity. When culture was finished in 10 days, *S. putrefaciens* IR-1 reduced 3.9 mM of Fe(III).

Discussion

A number of bacteria have been isolated and characterized based on their abilities to catalyze the dissimilatory reduction of metal ions including Fe(III) as their electron acceptor (18, 29, 27). Though the reduction ability of some microorganisms has been known earlier, much of metal reduction in nature environments was considered as the result of a non-enzymatic process (18). Recently, the reduction by microorganisms that can obtain energy for growth by the oxidation of organic compounds with a metal ion as the sole electron acceptor have been reported (18, 19, 22, 23, 27). It is widely recognized that the metal reducers play an important role in the cycling of metals and organic matters in sediments and other anaerobic environments (21). Our study also shows that Fe(III)-reduction is common in anaerobic environments. At the first enrichment culture, most samples showed similar Fe(III)-reduction activity while Fe(II) concentration increased as the enrichment culture was further performed. This result indicates that increasing Fe(II) concentration could be due to the increase of Fe(III)-reducer concentration.

Due to its lower solubility at neutral pH in aquatic environments, Fe(III)-reduction has been investigated by several researchers (18, 19, 27). They concluded that this reduction mechanism could be involved in electron carriers (i.e. cytochromes), which existed in the outer membrane of the cell (27). These proteins could directly transfer electrons, generated by the oxidation of the electron donor, to water-insoluble electron acceptor and Fe(III) was further reduced to Fe(II) (18, 19, 27). This suggestion was supported by our previous results that demonstrated that whole cells of *S. putrefaciens* IR-1 could directly contact the electrode without any electron mediators. In the previous study, the cyclic votammogram of bacterial whole cells showed typical peaks of cytochrome (13, 15). This result indicates that this organism possesses an electron transferring system on its outer membrane, and the outer membrane cytochromes are involved in this electron flow system (13, 15).

In spite of extensive studies, it is not clear as to the reason Fe(III)-reduction is coupled with energy conservation by electron transport phosporylation (ETP) (4, 20, 27). Table 2 shows that *S. putrefaciens* IR-1 fermented pyruvate without an electron acceptor but lactate was not. This result shows that this organism conserves energy by substrate level phosporylation with the oxidation of pyruvate. However, the oxidation of lactate as well as pyruvate was coupled with Fe(III)-reduction. In addition, bacterial cell growth was observed on the culture using these electron donors in the presence of Fe(III). Therefore, this organism is able to conserve ATP via ETP. However, the stoichiometric analysis was hindered by the interference from reactions such as a chemical reduction of Fe(III). In addition, some unidentified organic compounds were determined by HPLC when the organism was cultivated on lactate/Fe(III) (data not shown). In the future, further work is required to understand bacterial metabolism in the presence of Fe(III).

Acknowledgment

This work was supported by the Ministry of Science and Technology, Korea and KIST (2E15471). We are grateful to Mr Han-Woong Lee for the helpful guidance of the phylogenic relationship trees.

References

1. **Altschul, S.F., T.L. Madden, A.A. Schaffer, J.H. Zhang, Z. Zhang, W. Miller, and D.J. Lipman. 1997.** Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acid Res.* **25**, 3389-3402.
2. **Arnold, R.G., T.J. DiChristina, and M.R. Hoffmann.** 1988. Reductive dissolution of Fe(III) oxides by *Pseudomonas* species 200. *Biotechnol. Bioeng.* **32,** 1081-1096.
3. **Baumann, L., P. Baumann, M. Mandel, and R.D. Allen.** 1972. Taxonomy of aerobic marine eubacteria. *J. Bacteriol.* **110,** 402-429.
4. **Caccavo, F., D.J. Lonergan, D.R, Lovley, M. Davis, J.F. Stolz, and M.J. Mclnerney.** 1994. *Geobacter sulfurreducens* sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. *Appl. Environ. Microbiol.* **60,** 3752-3759.
5. **Canfield, D.E., B.B. Jorgenssen, H. Fossing, R. Flud, J. Gndersen, N.B. Ramsing, B. Thamdrup, J.W. Hansen, L.P. Nielsen, and P.O.J. Hall.** 1993. Pathways of organic carbon oxidation in three continental marine sediments. *Mar. Geol.* **113,** 27-40.

6. **Canfield, D., B. Thamdrup, and J.W. Hansen.** 1993. The anaerobic degradation of organic matter in Danish coastal sediments: Fe reduction, Mn reduction, and sulfate reduction. *Geochim. Cosmochim. Acta.* **57,** 3867-3883.
7. **Chang I.S., D.H. Kim, B.H. Kim, P.K. Shin, J.H. Yoon, J.S. Lee, and Y.H. Park.** 1997. Isolation and identification of carbon monoxide utilizing anaerobic bacteria, *Eubacterium limosum* KIST612. *Kor. J. Appl. Microbiol. Biotechnol.* **25,** 1-8.
8. **Chang, I.S., B.H. Kim, P.K. Shin, and W.K. Lee.** 1996. Bacterial contamination and its effects on ethanol fermentation. *J. Microbiol. Biotechnol.* **5,** 309-314.
9. **Coates, J.D., D.J. Lonergan, and D.R. Lovley.** 1995. *Desulfuromonas palmitatis* sp. nov., a marine dissimilatory Fe(III) reducer that can oxidize long-chain fatty acids. *Arch. Microbiol.* **164,** 406-413.
10. **Finster, K., and F. Bak.** 1993. Complete oxidation of propionate, valerate, succinate, and other organic compounds by newly isolated types of marine, anaerobic, mesophilic, gram-negative, sulfur-reducing eubacteria. *Appl. Environ. Microbiol.* **59,** 1452-1460.
11. **Ghiorse, W.C.** 1988. Microbial reduction of manganese and iron, p 305-331. *In* Zehnder , A. J. B. (ed.) Biology of anaerobic microorganisms. Wiley, New York.
12. **Herbert, D., P.J. Philips, and R.E. Strange.** 1971. Analysis of microbial cells, p 209-344. *In* J.R. Norris, and D.W. Ribbons (ed.) Methods in microbiology. Academic Press, New York.
13. **Kim, B.H., T. Ikeda, H.S. Park, H.J. Kim, M.S. Hyun, K. Kano, K. Takagi, and H. Tatsumi.** 1999. Electrochemical activity of an Fe(III)-reducing bacterium, *Shewanella putrifaciens* IR-1, in the presence of alternative electron acceptors. *Biotechnol. Tech.* **13,** 475-478.
14. **Kim. H.J., B.H. Kim, M.S. Hyun, and B.W. Cho.** 1998. Performance of a mediator-less microbial fuel cell using a metal-reducing bacterium, *Shewanella putrifaciens* IR-1. Abstr. O-12. p.395. *In* Abstracts of the 98th Gen. Meet. Am. Soc. Microb., Atlanta, American Society for Microbiology, Washington, D.C., U.S.A.
15. **Kim, B.H., H.J. Kim, M.S. Hyun, and D.H. Park.** 1999. Direct electrode reaction of Fe(III)-reducing bacterium, *Shewanella putrifaciens. J. Microbiol. Biotechnol.* **9,** 127-131.
16. **Laverman, A., J. Switzer Blum, J.K. Schaefer, E.J.P. Phillips, D.R. Lovley, and R. Oremland.** 1995. Growth of strain SES-3 with arsenate and other diverse electron acceptors. *Appl. Environ. Microbiol.* **61,** 3556-3561.
17. **Ljungdahl, L.G., and J. Wiegel.** 1982. Working with anaerobic bacteria, p. 115-127. *In* Demain A.L., and N.A. Solomon (eds.) Manual of Industrial Microbiology. American Society for Microbiology, Washington, D. C.
18. **Lovley, D.R.** 1987. Organic matter mineralization with the reduction of ferric iron: review. *Geomicrobiol. J.* **5,** 375-399.
19. **Lovley, D.R.** 1993. Dissimilatory metal reduction. *Annu. Rev. Microbiol.* **47,** 263-290.
20. **Lovley, D.R., S.J. Giovannoni, D.C. White, J.E. Champine, E.J.P. Phillips, Y.A. Gorby, and S. Goodwin.** 1993. *Geobacter metallireducens* gen. nov. sp. nov., a microorgnism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. *Arch. Microbiol.* **159,** 336-344.
21. **Lovley, D.R., and E.J.P. Phillips.** 1986. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl. Environ. Microbiol.* **51,** 683-689.
22. **Lovley, D.R., E.J.P. Phillips, and D.J. Lonergan.** 1989. Hydrogen and formate oxidation coupled to dissimilatory reduction of iron or manganese by *Altermonas putrefaciens. Appl. Environ. Microbiol.* **55,** 700-706.
23. **Lovley, D.R., E.J.P. Phillips, and D.J. Lonergan.** 1995. Fe(III) and S^{o} reduction by *Pelobacter carbinolicus. Appl. Environ. Microbiol.* **61,** 2132-2138.
24. **Lovley, D.R., J.C. Woodward, and F.H. Chapelle.** 1996. Rapid anaerobic benzene oxidation with a variety of chelated Fe(III) forms. *Appl. Environ. Microbiol.* **62,** 288-291.
25. **MacDonell, M.T., and R.R. Colwell.** 1985. Phylogeny of the Vibrionaceae and recommendation for two new genera, *Lisionella* and *Shewanella. Syst. Appl. Microbiol.* **6,** 171-182.
26. **McLaughlin, J.R., J.C. Ryden, and J.K. Syers.** 1981. Sorption of inorganic phosphate by iron- and aluminum-containing components. *J. Soil. Sci.* **32,** 365-377.
27. **Myers C.R., and K.H. Nealson.** 1990. Respiration-linked proton translocation coupled to anaerobic reduction of manganese(IV) and iron(III) in *Shewanella putrifaciens* MR-1. *J. Bacteriol.* **172,** 6232-6238.
28. **Schink, B., and M. Bomar.** 1991. The genera *Acetogenium, Acetoanaerobium* and *Acetitomaculum*. p. 1923-1936. *In* A. Balows, H.G. Trueper, M. Dworkin, W. Harder, and K.H. Schleifer (ed.) The Prokaryotes, 2nd ed. Springer Verlag. New York.
29. **Shewan, J.M., G. Hobbs, and W. Hodgkiss.** 1960. A determinative scheme for the identification of certain genera of Gram-negative bacteria with special reference to *Pseudomonadaceae. J. Appl. Bacteriol.* **23,** 379-390.
30. **Yoon, J.H., H.I. Kim, S.B. Kim, H.J. Kim, W.Y. Kim, S.T. Lee, M. Goodfellow, and Y.H. Park.** 1996. Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int. J. Syst. Bacteriol.* **46,** 502-506.