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Characterization of arsenate-, selenate- and nitrate-reducing activities in *Bacillus* sp. SF-1

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Abstract

A gram-positive and facultative anaerobic bacterium, *Bacillus* sp. SF-1, which was isolated from selenium-polluted sediment, can grow on lactate as an electron donor and selenate, arsenate or nitrate as an alternate electron acceptor in the absence of oxygen. This strain seems to be a promising agent for bioremediation of selenium/arsenic. In an effort to characterize the reductase systems in strain SF-1, arsenate, selenate and nitrate reduction activities were investigated with washed-cell suspensions and crude cell extracts from cells grown on arsenate, selenate and nitrate. These reductase activities were induced individually by the respective electron acceptors. Tungstate, which is a typical inhibitory antagonist of molybdenum-containing dissimilatory reductases, strongly inhibited reduction of arsenate, selenate and nitrate in the anaerobic growth cultures. These results suggested that strain SF-1 catalyzes the reduction of arsenate, selenate and nitrate by distinct terminal reductases containing molybdenum as a cofactor. This may be an advantage to utilize SF-1 in the bioremediation precesses where contaminants exist in mixture.

Key words: arsenate reduction, selenate reduction, nitrate reduction, *Bacillus* sp. SF-1, bioremediation

INTRODUCTION

Arsenic (As) and selenium (Se) are ubiquitous trace metalloids in the environment and required in trace amounts for growth and metabolism but are toxic elements at high concentrations. They are widely used in various industries including production of pesticides, medicinals, glass, pigments, and semiconductors[1, 2]. Consequently, industrial effluents can contain considerable amount of As and Se, and their contamination of many soils in Japan has been found primarily as a result of land investigation[3].

Metal or metalloid ion-reducing prokaryotes, which undertake respiration by reducing versatile electron acceptors under anaerobic conditions, have considerable implications for bioremediation of metal contamination[4, 5]. We isolated a selenate-reducing bacterium, *Bacillus* sp. SF-1, from Se-contaminated sediment[6]. Strain SF-1 reduced soluble selenate and selenite into insoluble elemental Se and utilized selenate as an electron acceptor for anaerobic respiration[6, 7]. A laboratory-scale continuous reactor using strain SF-1 had been constructed for removing toxic selenate and selenite from model wastewater, and both selenate and selenite were successfully reduced into nontoxic elemental Se and removed from the wastewater[8]. Recent works revealed that strain SF-1 was able to reduce

nitrate, nitrite, arsenate and tellurite in addition to selenate and selenite, and arsenate and nitrate seemed to act as terminal electron acceptors as well as selenate[7, 9]. Capability of dissimilatory arsenate reduction into arsenite implies that strain SF-1 may be utilized for extracting arsenic from contaminated soil for the purpose of bioremediation since arsenite is more mobile than arsenate[4, 10].

Although various dissimilatory arsenate and/or selenate-reducing prokaryotes have been isolated[11-21], only three different types of reductases, *i.e.* respiratory arsenate reductases from *Chrysiogenes arsenatis*[22] and *Bacillus selenitireducens*[23] and selenate reductase from *Thauera selenatis*[24], were purified and fully characterized. Selenate reduction by bacterial nitrate reductase has been also reported, while the affinity and the turnover rate for the selenate were much lower, as compared with those of selenate reductase from *T. selenatis*[25]. Thus, still little is known about the reducing enzyme systems in the prokaryotes, which are able to reduce arsenate, selenate and nitrate[11, 14, 26, 27] and a better understanding of the factors that regulate the synthesis and expression of the related enzymes in other selenate-/arsenate-reducing prokaryotes are required. Such information would be useful in the designing of engineered bioremediation processes utilizing such prokaryotes, especially when the target contaminants coexist with other alternative electron acceptors.

Here, we described the characterization of enzymatic systems in strain SF-1 for reduction of arsenate, selenate and nitrate using washed-cell suspensions and crude cell extracts. The results in this study support the conclusion that strain SF-1 is able to catalyze reduction of arsenate, selenate and nitrate by distinct terminal reductases, not by a single terminal reductase.

MATERIALS AND METHODS

Media and growth conditions The basal salt medium plus 0.1% yeast extract (BSMY) described previously was used for cultivation of strain SF-1[9]. For aerobic growth of strain SF-1, 10 g/*l* of glucose was added to BSMY as the sole carbon source, while 20 mM lactate was used instead of glucose for anaerobic growth with one of the following electron acceptors: arsenate, selenate or nitrate. Liquid cultures were prepared in 50-m*l* serum bottles containing 20 m*l* of medium or 500-m*l* Erlenmeyer flasks containing 500 m*l* of medium. For aerobic cultivation, bottles were plugged with cotton. For anaerobic cultivation, bottles were sealed with butyl rubber septums and aluminum crimp seals, and the head space of the bottles was filled with N_2 gas. Bacterial cultures were incubated on a rotary shaker (120 rpm, 30℃) in the dark condition.

Experiments with washed-cell suspensions Log-phase cells of strain SF-1 grown with arsenate (10 mM), selenate (10 mM), or nitrate (10 mM) as the sole electron acceptor in the anaerobic cultures were harvested by centrifugation (6,000 × *g*, 10 min, 4℃). Cells aerobically grown (*i.e.*, oxygen as the electron acceptor) were also harvested. The harvested cells were washed with 50 mM Tris-HCl buffer (pH8.0) twice and suspended in the same buffer containing lactate (5 mM) and arsenate (1 mM), selenate (1 mM), or nitrate (1 mM) to test for the ability to reduce them as an electron acceptor. Cell suspensions (20 m*l*) were incubated in 50-m*l* serum bottles with N_2 atmosphere on a rotary shaker (120 rpm, 30℃). The selenate-grown cells were tested for the ability to reduce selenate and nitrate simultaneously in the suspension including selenate (1 mM) and nitrate (1 mM). To investigate whether the reducing-activity of cells were induced during experiments with washed-cell suspensions, the cell suspensions were incubated with 20 mg/*l* chloramphenicol (Cm) to arrest the protein synthesis. Bottles were periodically sacrificed, and portion of suspensions was centrifuged. After centrifugation supernatants were frozen as samples until analyses have done. All experiments were performed in duplicate and the results are shown as the average values (difference of the data obtained in the duplicate experiments was within 5%).

Preparation of crude cell extracts and enzyme assay Cells of strain SF-1 were

grown anaerobically in 1-*l* cultures (two 500-m*l* Erlenmeyer flasks) with arsenate (10 mM), selenate (10 mM), or nitrate (10 mM) as the sole electron acceptor until late log phase. Cells were harvested by centrifugation and washed twice with 50 m*l* ice-cold 50 mM Tris-HCl buffer (pH 8.0) containing 1mM dithiothreitol (DTT) (Buffer A). The cells resuspended in 10 m*l* ice-cold Buffer A containing 1mM phenylmethane sulfonyl fluoride were then disrupted by passing through a French press (9.8×10^7 Pa, Thermo Electron Corp., MA, USA). After DNase and RNase treatment, unbroken cells were removed by centrifugation at 2,600 × *g* for 5 min at 4℃ and the supernatants were used as crude cell extracts. Assay of dissimilatory arsenate reductase, selenate reductase, and nitrate reductase was performed as described previously[22, 28], by measuring the oxidation of the reduced benzyl viologen as an artificial electron donor, and the activity was calculated as one μmol of benzyl viologen oxidized per min using the extinction coefficient of 19.5 cm^{-1} mM^{-1}. One unit (U) of activity was defined as the activity associated with the reduction of 1 μmol of arsenate, selenate, or nitrate per min at 30℃, pH 7.4.

Inhibition experiment with tungstate To investigate whether arsenate, selenate and nitrate reductases in strain SF-1 can contain molybdenum cofactor, the effect of tungstate on arsenate, selenate and nitrate reduction was tested in anaerobic growth cultures. Log-phase cells of strain SF-1 cultivated aerobically were inoculated into lactate-BSMY containing arsenate (1 mM), selenate (1 mM) or nitrate (1 mM). Cultures (20 m*l*) were incubated with 1 mM tungstate in anaerobic (N_2 atmosphere) serum bottles and reducing activities were measured after 4 or 8 h.

Analytical procedures Cell density of strain SF-1 in cell suspensions was determined by microscopic direct cell counting with a counting chamber. Arsenate, selenate and selenite were determined by ion chromatography with conductivity detector[9]. Nitrate and nitrite were measured by ion chromatography[9] equipped with SPD-10AV UV-VIS detector (Shimazu Corp., Kyoto) instead of conductivity detector.

RESULTS

Arsenate reduction by washed cell suspensions Washed cells of strain SF-1 grown on arsenate, selenate, nitrate and oxygen (aerobically grown) as the electron acceptor, respectively, were examined for the ability to reduce arsenate. Arsenate-grown cells of strain SF-1 actively reduced arsenate, of which 1 mM was almost completely reduced within 8 hours, whereas selenate-, nitrate- and oxygen-grown cells were not able to reduce arsenate significantly (Table 1). No activity was shown in control experiments without any electron donor (data not shown).

Selenate reduction by washed-cell suspensions Capability of selenate reduction was tested using the washed-cell suspensions as same method as that of arsenate reduction. Selenate reduction was observed only in the washed-cell grown on selenate (Table 2). Neither selenate reduction nor selenite accumulation was observed in the cell suspensions grown on arsenate, nitrate and oxygen as the electron acceptor. The consumption of selenate using selenate-grown cells of strain SF-1 matched approximately the accumulation of selenite (data not shown). Although strain SF-1 was

Table 1 Arsenate-reducing activity in arsenate-, selenate-, nitrate-, and oxygen (aerobic)-grown washed-cell suspensions of strain SF-1 with lactate as electron donor[a]

Cell suspensions	Reduced arsenate (%)[b] after		Cell density[c] (×10^7 cells/m*l*)
	4 h	8 h	
Arsenate-grown	62.4	98.2	3.3
Selenate-grown	0	8.1	3.0
Nitrate-grown	0	0	1.6
Oxygen-grown	5.2	0	2.8

[a] Initial lactate concentration was 5 mM, arsenate concentration was 1 mM.
[b] Results were expressed as the percentage of reduced arsenate after 4 h or 8 h incubation. Initial percentage before incubation was considered as 0 %.
[c] Cell density indicates cell number per ml of the suspension. Cell growth was not observed during incubation.

Table 2 Selenate-reducing activity in arsenate-, selenate-, nitrate-, and oxygen (aerobic)-grown washed-cell suspensions of strain SF-1 with lactate as electron donor[a]

Cell suspensions	Reduced selenate (%)[b] after		Cell density[c] (×10⁷ cells/ml)
	4 h	8 h	
Arsenate-grown	0.5	0.4	1.3
Selenate-grown	5.1	13.7	1.5
Nitrate-grown	2.2	0	1.2
Oxygen-grown	0.5	0	2.8

[a] Initial lactate concentration was 5 mM, selenate concentration was 1 mM.
[b] Results were expressed as the percentage of reduced selenate after 4 h or 8 h incubation. Initial percentage before incubation was considered as 0 %.
[c] Cell density indicates cell number per ml of the suspension. Cell growth was not observed during incubation.

Table 3 Nitrate-reducing activity in arsenate-, selenate-, nitrate-, and oxygen (aerobic)-grown washed-cell suspensions of strain SF-1 with lactate as the electron donor[a]

Cell suspensions	Reduced nitrate (%)[b] after		Cell density[c] (×10⁷ cells/ml)
	4 h	8 h	
Arsenate-grown	3.2	0.9	1.3
Selenate-grown	16.6	43.6	3.7
Selenate-grown with Cm[d]	11.7	30.2	3.7
Nitrate-grown	35.8	49.1	3.9
Oxygen-grown	8.3	19.7	2.8
Oxygen-grown with Cm[d]	6.2	2.1	3.9

[a] Initial lactate concentration was 5 mM, nitrate concentration was 1 mM.
[b] Results were expressed as the percentage of reduced nitrate after 4 h or 8 h incubation. Initial percentage before incubation was considered as 0 %.
[c] Cell density indicates cell number per ml of the suspension. Cell growth was not observed during incubation.
[d] Cell suspensions were incubated with 20 mg/l chloramphenicol (Cm).

able to reduce selenite to elemental selenium in anaerobic growth cultures[6], further reduction of selenite accumulated was not observed in washed-cells grown on selenate.

Nitrate reduction by washed-cell suspensions Nitrate-reducing activity was also investigated using the washed-cell suspensions. In the suspensions containing nitrate, the selenate- and oxygen-grown cells reduced nitrate as well as the nitrate-grown cells did (Table 3). Further reduction of nitrite accumulated that resulted from nitrate reduction was not observed in the selenate-, nitrate- and oxygen-grown cell suspensions, while strain SF-1 immediately reduced nitrite to ammonia in anaerobic growth experiment (data not shown). Arsenate-grown cells could neither reduce nitrate clearly (Table 3) nor accumulate nitrite (data not shown). Cm completely inhibited the reduction activity of nitrate in the oxygen-grown cells, while did mildly in the selenate-grown cells (Table 3).

Simultaneous selenate and nitrate reduction by the selenate-grown cells To obtain detailed information for the enzymatic systems relevant to selenate and nitrate reduction in strain SF-1, the selenate-grown cells, which were capable of both selenate (Table 2) and nitrate reduction (Table 3), were incubated in the co-presence of selenate and nitrate with or without Cm (Fig. 1). In the cell suspensions, Cm had no noticeable effect upon selenate reduction (Fig. 1A). On the other hand, the curve of nitrate reduction with Cm was slowed down, as compared with that without Cm, and reached flat after 8 h (Fig. 1B). In the absence of Cm, the reduction of nitrate proceeded continuously and the reduction of selenate and nitrate was virtually simultaneous (Fig. 1A, B).

Reductase activities in crude cell extracts To determine the dissimilatory arsenate, selenate and nitrate reductase activities in strain SF-1, crude cell extracts from cells grown on arsenate, selenate, or nitrate as the sole electron acceptor were tested for the ability to couple the oxidation of benzyl viologen with the reduction of each electron acceptor. Reductase activities in each cell extract were presented in Table 4. Crude extracts from cells grown on arsenate as an electron acceptor exhibited the highest reductase activity for arsenate. As similarly, selenate-grown crude cell extracts showed highest reductase activity for selenate, and nitrate-grown crude cell extracts for nitrate, respectively. The maximum reductase

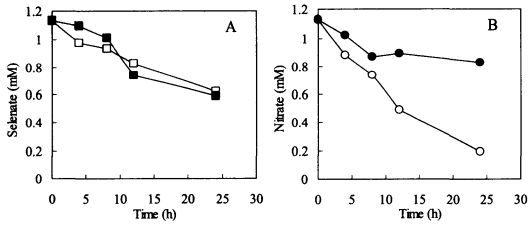

Fig. 1 Effect of chloramphenicol (Cm) on the selenate (A) and nitrate (B) reduction by selenate-grown cell suspensions in the co-presence of both oxyanions. Cell suspensions containing 5 mM lactate, 1 mM selenate and 1 mM nitrate were incubated with 20 mg/l Cm (solid symbols) or without (open symbols) . Symbols: squares, selenate concentration; circles, nitrate concentration

Table 4 Comparison of the reductase activity in the different crude cell extracts with each of terminal electron acceptors[a]

Crude extracts from cells grown on:	Enzyme activity (%)[b] for each of electron acceptors		
	Arsenate	Selenate	Nitrate
Arsenate	100[c]	7	2
Selenate	27	100[d]	3
Nitrate	2	1	100[e]

a The enzyme activity was measured as benzyl viologen (artificial electron donor) oxidized per min.
b The percent enzyme activity was calculated by giving a value of 100% to the enzyme activity of each crude cell extracts on its specific substrate. Each value represents an average of two analyses (difference of the data obtained in the two analyses was within 2%).
c The activity of arsenate-grown cell extracts with arsenate was 47.3 U.
d The activity of selenate-grown cell extracts with selenate was 17.6 U.
e The activity of nitrate-grown cell extracts with nitrate was 48.4 U.

activity in a given crude cell extract was obtained against the substrate, on which the cells were grown. The least substrate specificity was observed in crude extracts from selenate-grown cells; the enzyme activity for arsenate was 27% of that for selenate (Table 4).

Inhibition of arsenate, selenate and nitrate reduction by tungstate Effects of tungstate on the arsenate, selenate and nitrate reduction were investigated in anaerobic growth experiments (Fig. 2). In the absence of tungstate, strain SF-1 actively reduced 1 mM

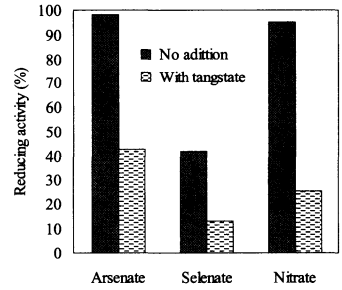

Fig. 2 Effect of tungstate on the arsenate, selenate and nitrate reduction in anaerobic growth experiments. Cultures were incubated with 20 mM lactate and 1 mM arsenate, 1 mM selenate or 1 mM nitrate in the presence or absence of 1 mM tungstate. Reducing activity was expressed as the percentage of initial arsenate, selenate or nitrate reduced after 4 h (nitrate) or 8 h (arsenate, selenate) incubation

arsenate, selenate and nitrate, especially, arsenate and nitrate were completely reduced within 8 h and 4 h, respectively. However, addition of tungstate (1 mM) lowered significantly the activities of arsenate, selenate and nitrate reduction. The ratio of inhibition for arsenate, selenate and nitrate reduction was 56.7%, 69.7% and 73.8%, respectively. Therefore, tungstate appeared to strongly inhibit all of three reduction activities.

DISCUSSION

In this study, we examined the capability of strain SF-1 to reduce arsenate, selenate and nitrate in washed-cell suspensions when grown on arsenate, selenate, nitrate and oxygen as the electron acceptor. Arsenate- and selenate-reducing activities were found only in arsenate- and selenate-grown washed cells, respectively. These results presumably indicated that arsenate and selenate induced the synthesis of corresponding reductases in strain SF-1, respectively. On the other hand, nitrate-reducing activity was found not only in nitrate-grown cells but also in selenate- and oxygen-grown cells, whereas arsenate-grown cells had no nitrate-reducing activity. Nitrate reduction of selenate- and oxygen-grown cells were inhibited by addition of protein synthesis inhibitor, Cm, suggesting that nitrate reductase was induced during experiments in the washed-cell suspensions

by the presence of nitrate. Although nitrate-reducing activity in selenate-grown cells was not completely inhibited even if Cm was added, the competitive inhibition of selenate and nitrate reduction did not occur in selenate-grown cell suspensions containing both oxyanions, and Cm inhibited significantly only nitrate reduction. Therefore, it must be clear that the reduction of selenate and nitrate was catalyzed by separate reduction system; a selenate reductase and a nitrate reductase. Thus, these results indicate that strain SF-1 synthesizes separate inducible enzymes, dissimilatory arsenate reductase, selenate reductase and nitrate reductase, which are specifically induced by the corresponding oxyanions, to utilize arsenate, selenate and nitrate, respectively, as the terminal electron acceptors.

The results of enzyme assays using crude cell extracts, *i.e.*, the highest reductase activities for arsenate, selenate and nitrate were obtained from crude cell extracts grown on arsenate, selenate, and nitrate as the electron acceptor, respectively, strongly supported this conclusion. In this regard, however, crude cell extracts of selenate-grown cells slightly exhibited the reductase activity for arsenate. Although each reductase complex has not been purified yet, it is possible that selenate reductase of strain SF-1 has somewhat broader substrate specificity compared with arsenate and nitrate reductases. However, this reductase activity, which was detected by the measuring the oxidation of benzyl viologen, may not be physiologically relevant as washed-cell suspensions of strain SF-1 grown on selenate were not able to reduce arsenate

Interestingly, there seems to be significant different reductase system between strain SF-1 and other prokaryotes that can reduce arsenate, selenate and nitrate as the electron acceptors. Washed-cell suspensions of both selenate- and nitrate-grown cells of *Sulfurospirillum barnesii* had a constitutive ability to reduce arsenate, although the arsenate-grown cells catalyzed selenate reduction[26, 29]. The selenate-grown washed cells from *Bacillus arsenicoselenatis* also showed a constitutive capability of reducing arsenate[27], while a constitutive selenate reduction was not observed[26]. In this way, it is possible that their selenate and/or nitrate reductases can reduce arsenate. By contrast, arsenate reduction in strain SF-1 was catalyzed only by an inducible arsenate reductase, which is clearly distinct from selenate and nitrate reductases.

Tungstate, which blocked a number of molybdo-enzymes including nitrate reductase by substituting tungsten for molybdenum at the active site[30-32], had strong inhibitory effects upon arsenate, selenate and nitrate reduction in anaerobic growth condition. Therefore, the dissimilatory arsenate and selenate reductases in strain SF-1 seemed to contain molybdenum as a cofactor as well as the dissimilatory arsenate reductase of *C. arsenatis*[19] and *B. selenitireducens*[20], and selenate reductase of *T. selenatis*[21].

CONCLUSIONS

The presented studies strongly suggest that strain SF-1 possesses separate pathways for the dissimilatory reduction of arsenate, selenate and nitrate. Therefore, since good cell growth occurred in the anaerobic growth cultures containing nitrate, as compared with that containing arsenate or selenate (data not shown), the presence of small amount of nitrate will improve the arsenate and selenate reduction rate in bioreactors treating contaminated soils and wastewater without competitive inhibition on the reduction of target oxyanions. Although previous works suggested that a high concentration of nitrate prevented selenate reduction[7], selenate and nitrate can be reduced simultaneously by inducing selenate reductase. Thus, controlling the expression of the reductases may lead to effective removal of target contaminants even in the presence of alternative electron acceptors. From such a viewpoint, strain SF-1 seems to have an advantage as a bioremedial agent for metal contamination. In addition, purification and characterization of the specific arsenate, selenate and nitrate reductase based on these findings can accelerate the biological understanding of this strain and ultimately facilitate the development of its bio-remediation potential.

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REFERENCES

1) **Azcue, J. M. and Nriagu, J. O.**: Arsenic: historical perspectives., *In* Nriagu, J. O. (ed.), Arsenic in the environment, partI: cycling and characterization., Willy-Interscience, p. 1-16, New York (1994)
2) **Haygarth, P. M.**: Global importance and global cycling of selenium., *In* Frankenberger, W. T., Jr. and Benson, S. (ed.), Selenium in the environment., Marcel Dekker Inc., p. 1-27, New York (1994)
3) **Ministry of the Environment of Japan**: Overview of survey results about investigation and measures for soil pollution, and present situation., http://www.env.go.jp/water/report/h14-01/index.html, (2002) (in Japanese)
4) **Lovley, D. R. and Coates, J. D.**: Bioremediation of metal contamination., Curr. Opin. Biotech., 8, 285-289 (1997)
5) **Gadd, G. M.**: Bioremedial potential of microbial mechanisms of metal mobilization and immobilization., Curr. Opin. Biotechnol., 11, 271-279 (2000)
6) **Fujita, M., Ike, M., Nishimoto, S., Takahashi, K., and Kahiwa, M.**: Isolation and characterization of a novel selenate-reducing bacterium, *Bacillus* sp. SF-1., J. Ferment. Bioeng., 83, 517-522 (1997)
7) **Kashiwa, M., Nishimoto, S., Takahashi, K., Ike, M., and Fujita, M.**: Factors affecting soluble selenium removal by a selenate-reducing bacterium *Bacillus* sp. SF-1., J. Biosci. Bioeng., 89, 528-533 (2000)
8) **Fujita, M., Ike, M., Kashiwa, M., Hashimoto, R., and Soda, S.**: Laboratory-scale continuous reactor for soluble selenium removal using selenate-reducing bacterium, *Bacillus* sp. SF-1., Biotechnol. Bioeng., 80, 755-761 (2002)
9) **Yamamura, S., Ike, M, and Fujita, M.**: Dissimilatory arsenate reduction by a facultative anaerobe, *Bacillus* sp. strain SF-1., J. Biosci. Bioeng., 96, 454-460 (2003)
10) **Dowdle, P. R., Laverman, A. M., and Oremland, R. S.**: Bacterial dissimilatory reduction of arsenic(V) to arsenic(III) in anoxic sediments., Appl. Environ. Microbiol., 62, 1664-1669 (1996)
11) **Stolz, J. F. and Oremland, R. S.**: Bacterial respiration of arsenic and selenium., FEMS Miclobiol. Rev., 23, 615-627 (1999)
12) **Macy, J. M., Santini, J. M., Pauling, B. V., O'Neill, A. H., and Sly, L. I.**: Two new arsenate/sulfate-reducing bacteria: mechanisms of arsenate reduction., Arch. Microbiol., 173, 49-57 (2000)
13) **Blum, J. S., Stolz, J. F., Oren, A., and Oremland, R. S**: *Selenitihalanaerobacter shriftii* gen. nov., sp. nov., a halophilic anaerobe from Dead Sea sediments that respires selenate., Arch. Microbiol., 175, 208-219 (2001)
14) **Niggemyer, A., Spring, S., Stackebrandt, E., and Rosenzweig, R. F.**: Isolation and characterization of a novel As(V)-reducing bacterium: implication for arsenic mobilization and the genus *Desulfitobacterium*., Appl. Environ. Microbiol., 67, 5568-5580 (2001)
15) **Gihring, T. M. and Banfield, J. F.**: Arsenite oxidation and arsenate respiration by a new *Thermus* isolate., FEMS Microbiol. Lett., 204, 335-340 (2001)
16) **Santini, J. M., Stolz, J. F., and Macy, J. M.**: Isolation of a new arsenate-respiring bacterium-physiological and phylogenetic studies., Geomicrobiol. J., 19, 41-52 (2002)
17) **Herbel, M. J., Blum, J. S., Hoeft, S. E., Cohen, S. M., Arnold, L. L., Lisak, J., Stolz, J. F., and Oremland, R. S.**: Dissimilatory arsenate reductase activity and arsenate-respiring bacteria in bovine rumen fluid, hamster feces, and the termite hindgut., FEMS Microbiol. Ecol., 41, 59-67 (2002)
18) **Stolz, J. F., Basu, P., and Oremland, R. S.**: Microbial transformation of elements: the case of arsenic and selenium., Int. Microbiol., 5, 201-207 (2002)
19) **Saltikov, C. W., Cifuentes, A.,**

Venkateswaran, K., and Newman, D. K.: The *ars* detoxification system is advantageous but not required for As(V) respiration by the genetically tractable *Shewanella* species strain ANA-3., Appl. Environ. Microbiol., 69, 2800-2809 (2003)

20) **Oremland, R. S. and Stolz, J. F.**: The ecology of arsenic., Science, 300, 939-944 (2003)

21) **Takai, K., Kobayashi, H., Nealson, K. H., and Horikoshi, K.**: *Deferribacter desulfuricans* sp. nov., a novel sulfer-, nitrate- and arsenate-reducing thermophile isolated from a deep-sea hydrothermal vent., Int. J. Syst. Evol. Microbiol., 53, 839-846 (2003)

22) **Kraft, T. and Macy, J. M.**: Purification and characterization of the respiratory arsenate reductase of *Chrysiogenes arsenatis*., Eur. J. Biochem., 255, 647-653 (1998)

23) **Afkar, E., Lisak, J., Saltikov, C., Basu, P., Oremland, R. S., and Stolz, J. F.**: The respiratory arsenate reductase from *Bacillus selenitireducens* strain MLS10., FEMS Microbiol. Lett., 226, 107-112 (2003)

24) **Schröder, I., Rech, S., Krafft, T., and Macy, J. M.**: Purification and characterization of the selenate reductase from *Thauera selenatis*., J. Biol. Chem., 272, 23765-23768 (1997)

25) **Sabaty, S., Avazeri, C., Pignol, D., and Vermeglio, A.**: Characterization of the reduction of selenate and tellurite by nitrate reductase., Appl. Environ. Microbiol., 67, 5122-5126 (2001)

26) **Oremland, R. S., Blum, J. S., Bindi, A. B., Dowdle, P. R., Herbel, M., and Stolz, J. F.**: Simultaneous reduction of nitrate and selenate by cell suspensions of selenium-respiring bacteria., Appl. Environ. Microbiol., 65, 4385-4392 (1999)

27) **Blum, J. S., Bindi, A. B., Buzzelli, J., Stolz, J. F., and Oremland, R. S.**: *Bacillus arsenicoselenatis*, sp. nov., and *Bacillus selenitireducens*, sp. nov.: two haloalkaliphiles from Mono Lake, California that respire oxyanions of selenium and arsenic., Arch. Microbiol., 171, 19-30 (1998)

28) **Stolz, J. F., Gugliuzza, T., Blum, J. S., Oremland, R., and Murillo, F. M.**: Differential cytochrome content and reductase activity in *Geospirillum barnesii* strain SeS3., Arch. Microbiol., 167, 1-5 (1997)

29) **Laverman, A. M., Blum, J. S., Schaefer, J. K., Philips, E. J. P., Lovley, D. R., and Oremland, R. S.**: Growth of strain SES-3 with arsenate and other diverse electron acceptors., Appl. Environ. Microbiol., 61, 3556-3561 (1995)

30) **Prins, R. A., Cline-Theil, W., Malestein, A., and Counotte, G. H. M.**: Inhibition of nitrate reduction in some rumen bacteria by tungstate., Appl. Environ. Microbiol., 40, 163-165 (1980)

31) **Chauret, C. and Knowles, R.**: Effect of tungstate on nitrate and nitrite reductases in *Azospirillum brasilense* Sp7., Can. J. Microbiol., 37, 744-750 (1991)

32) **Gates, A. J., Hughes, R. O., Sharp, S. R., Millington, P. D., Nilavongse, A., Cole, J. A., Leach, E.-R., Jepson, B., Richardson, D. J., and Butler, C. S.**: Properties of the periplasmic nitrate reductases from *Paracoccus pantotrophus* and *Escherichia coli* after growth in tungsten-supplemented media., FEMS Microbiol. Lett., 220, 261-269 (2003)

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