Journal of Industrial Microbiology (1995) 14, 329–336

Transformation of selenate and selenite to elemental selenium by *Desulfovibrio desulfuricans*

Francisco A. Tomei[1], Larry L. Barton[2], Cheryl L. Lemanski[3], Thomas G. Zocco[4], Nancy H. Fink[3] and Laurel O. Sillerud[3]

[1]*Army Environmental Policy Institute, Georgia Institute of Technology, 430 Tenth Street, NW Suite S-205, Atlanta, GA 30318,* [2]*Laboratory of Microbial Chemistry, Department of Biology, University of New Mexico, Albuquerque, NM 87131,* [3]*Life Science Division, Los Alamos National Laboratory, Los Alamos, NM 87545 and* [4]*Physical Metallurgical Group, Material Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545, USA*

(Received 2 May 1994; accepted 22 October 1994)

Key words: Selenium; *Desulfovibrio*; Sulfate-reducing bacteria

SUMMARY

Desulfovibrio desulfuricans (DSM 1924) can be adapted to grow in the presence of 10 mM selenate or 0.1 mM selenite. This growth occurred in media containing formate as the electron donor and either fumarate or sulfate as the electron acceptor. As determined by electron microscopy with energy-dispersive X-ray analysis, selenate and selenite were reduced to elemental selenium which accumulated inside the cells. Selenium granules resulting from selenite metabolism were cytoplasmic while granules of selenium resulting from selenate reduction appeared to be in the periplasmic region. The accumulation of red elemental selenium in the media following stationary phase resulted from cell lysis with the liberation of selenium granules. Growth did not occur with either selenate or selenite as the electron acceptor and ^{13}C nuclear magnetic resonance indicated that neither selenium oxyanion interfered with fumarate respiration. At 1 μM selenate and 100 μM selenite, reduction by *D. desulfuricans* was 95% and 97%, respectively. The high level of total selenate and selenite reduced indicated the suitability of *D. desulfuricans* for selenium detoxification.

INTRODUCTION

Selenium oxyanions in the environment are of concern because oxidized forms of selenium at elevated concentrations are highly toxic to living systems [27]. As analogues to sulfate and sulfite, selenate and selenite interact with enzymes of assimilatory sulfate reduction. Selenate inhibits sulfate transport systems in bacteria [8,9,19,23,28] while selenite inhibits sulfite reductases [18,20]. Additionally, the oxyanions of selenium inhibit dissimilatory sulfate reduction in *Desulfovibrio* and *Desulfotomaculum* [25,30].

Several different bacteria reduce selenate or selenite to elemental selenium [22,35]. One mechanism for selenium detoxification involves glutathionine and glutathionine reductase [6]. Since glutathionine is not at a detectable level in *Desulfovibrio* [12], the formation of elemental selenium initially reported by Postage [30] with strains of sulfate-respiring bacteria may be expected to be attributed to a chemical reaction involving hydrogen sulfide. At least one patient has been filed to exploit the use of sulfate-reducing bacteria in detoxification of selenate in mine wastes [2].

With the report [24] that selenate serves as an electron acceptor for a new bacterial isolate, it became important to examine selenium metabolism by *Desulfovibrio*. In this report we provide evidence that the transformation of selenate or selenite by *D. desulfuricans* can occur under conditions where metabolism has no influence on electron acceptor activity by the culture. The information presented in this paper helps define the conditions under which sulfate-reducing bacteria can participate in selenium detoxification in a sulfate-enriched environment.

Correspondence to: Dr Larry L. Barton, Department of Biology, University of New Mexico, Albuquerque, NM 87131-1091, USA.

MATERIALS AND METHODS

Growth

D. desulfuricans subsp. *desulfuricans* (DSM 1924), obtained from the German Culture Collection in Braunschweig, was grown in a defined medium that contained the following: 4.71 g $NaHCO_3$, 0.8 g NaH_2PO_4, 0.4 g KH_2PO_4, 238 mg NH_4Cl, 83 mg $CaCl_2$, 102 mg $MgCl_2 \cdot 6H_2O$, 20 mg $FeCl_2 \cdot 4H_2O$, 1 mg Na_2EDTA, 136 mg sodium acetate, 1 ml of metal mixture, 10 ml of a vitamin solution and 1 L of distilled water. The metal mixture contained in 1 L: 0.5 g Na_2EDTA, 0.5 g $MnCl_2 \cdot 4H_2O$, 50 mg H_3BO_3, 50 mg $ZnCl_2$, 50 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 50 mg $AlCl_3$, $CoCl_2 \cdot 6H_2O$, 50 mg $NiCl_2 \cdot 6H_2O$, 30 mg $CuCl_2 \cdot 2H_2O$. The vitamin solution contained the following in 1 L: 5 mg pyridoxine, 5 mg thiamine, 5 mg riboflavin, 5 mg niacinamide, 5 mg D-pantothenic acid, 5 mg *p*-aminobenzoic acid, 5 mg D,L-α-lipoic acid, 5 mg cyanocobalamine, 2 mg biotin, and 2 mg folic acid. The electron donor was 40 mM sodium formate while the electron acceptor was either 40 mM disodium fumarate or 40 mM sodium sulf-

ate. Ten milliliters of the filter-sterilized medium was placed in 27-ml serum tubes and 80% Ar/20% CO_2 was added under two atmospheres of pressure. At equilibrium, the pH of the medium was 6.5. Ten microliters of an active culture was used as an inoculum and the optical density was followed at 580 nm using a Bausch and Lomb Spectronic 20 (Bausch and Lomb Inc., Rochester, NY, USA).

Nuclear Magnetic Resonance (NMR) spectroscopy

Proton-decoupled ^{13}C-NMR spectra were accumulated with the aid of a Bruker AM400wb spectrometer system (Bruker Instruments, Inc., Billerica, MA, USA) operated at a carbon frequency of 100.61 MHz, using a sweep width of 22 727 Hz in 16 K data points, and operated at an acquisition time of 0.36 s. A relaxation delay of 0.7 s was applied after each 16 μs (60°) pulse. Bi-level decoupling (0.5–4 Watts) was used to avoid sample heating. The 20-mm spinning sample tubes were maintained at 25 °C. A 10-Hz exponential was applied before Fourier transformation. The chemical shifts were referenced to tetramethylsilane by applying the spectral-reference frequency for the β-anomeric carbon of glucose to 97.3 p.p.m. [7].

^{77}Se was followed using natural abundance in commercially available selenate and selenite using established procedures [26]. Sample processing was as described for ^{13}C NMR analysis. Standardization was accomplished using 1 M solutions of sodium selenate and sodium selenite. To ensure detection of inorganic selenium compounds in solution, data from sample tubes was collected over a period of several hours.

Determination of selenium

To measure selenium in the reactions, a fluorimetric procedure using 2,3-diaminonaphthalene as an essential reagent [32] was employed. Cells to be analyzed were collected on filters (Millipore Corp., Bedford, MA, USA) with a pore diameter of 0.2 μm and the filters were washed three times with 0.85% NaCl to remove soluble selenium species. These filters were placed in acid-cleaned test tubes to be digested with perchloric acid and nitric acid as previously described [32]. Limits of sensitivity for this assay system under the conditions employed was 5 ng selenium.

Electron microscopy

Whole mounts were prepared by withdrawing aliquots with a tuberculin syringe and placing a drop on a TEM grid coated with formvar. The sample was dried at room temperature and grids were coated with carbon at this time. For sections, the sample was fixed in 2.5% glutaraldehyde in 0.1 M HEPES buffer at pH 7.6, dried with ethanol and embedded in a low velocity resin using the procedures previously published [33]. A light carbon film was deposited over the sectioned specimen to preserve selenium granules while the specimen was exposed to the electron beam. Cells were examined with a JEOL 2000EX scanning-transmission electron microscope (JEOL USA Inc., Peabody, MA, USA) and elemental analysis was achieved with a energy-dispersive X-ray analysis using a Tracor Northern 5500 spectrophotometer system (Tracor Northern, Inc., Middleton, WI, USA).

RESULTS

Growth response

The growth of *D. desulfuricans* in formate/fumarate media containing selenate or selenite are presented in Fig. 1. The lag phase of growth was extended as the amount of selenate or selenite was increased in the media. While a lag period of over 30 h was observed with media containing selenate, a lag phase of only a few hours was noted with selenite added to the media. Cell yields in selenium-containing media was comparable to controls receiving no additions of selenate or selenite. Cultures grown in 100 μM selenite produced elemental selenium at a time earlier than cultures exposed to selenate. The appearance of a red color in the medium (arrows in Fig. 1(B)) was suggestive of the formation of elemental selenium and this was preceeded by the cessation of growth.

When *D. desulfuricans* was grown with sulfate as the electron acceptor (Fig. 1(C and D)), cultures attained a higher maximum cell yield than those cultures grown in fumarate-containing media. This phenomenon was not related to selenate or selenite presence because greater cell yields were obtained with sulfate as compared to fumarate as the electron acceptor in cultures receiving no selenium. Cultures of *D. desulfuricans* exposed to selenate and grown with sulfate as the electron acceptor did not show the lag phase observed in cultures grown in fumarate media containing selenate. *D. desulfuricans* grown in 1 μM selenate grew at the same rate and reached the same cell yield as cultures grown in media to which selenate was not added. Cells inoculated into formate/sulfate medium with 10 μM selenate grew at a rate slower than control cultures. The growth rate for cultures in 1 μM selenate was lower than in selenate-containing media.

Evidence was found for an inorganic reaction between sulfide produced by metabolizing bacteria and selenite added to the media. A pale orange color developed at about 50 h after inoculation with *D. desulfuricans* in a tube containing 0.1 mM selenite at a time when no sign of turbidity was evident (left arrow in Fig. 1(D)). Bacterial growth was detected in this tube after the color developed and continued to increase (right arrow in Fig. 1(D)). Because of these chemical artifacts additional experiments were conducted only with fumarate as the electron acceptor.

Cells transferred from cultures growing in 0.1 mM selenite or selenate were used for a series of growth experiments. With the inoculum taken from a culture grown in selenate medium, increased resistance to selenate (Fig. 2) relative to unadapted cultures (Fig. 1) was observed. These cultures with inocula from adapted cultures grew without a lag phase but continued to show slower rates and cell yields than controls receiving no selenate. Cultures failed to grow in 1 mM or 10 mM selenite when the inoculum used was taken from cultures adapted to 0.1 mM selenite or 10 mM selenate. To maintain cultures of *D. desulfuricans* adapted to selenate or selenite, it was necessary to subculture no later than when the culture entered the stationary phase.

Several different growth patterns were obtained when *D. desulfuricans* were grown in media containing 0.1 mM cysteine in addition to selenate or selenite. Cultures grew in

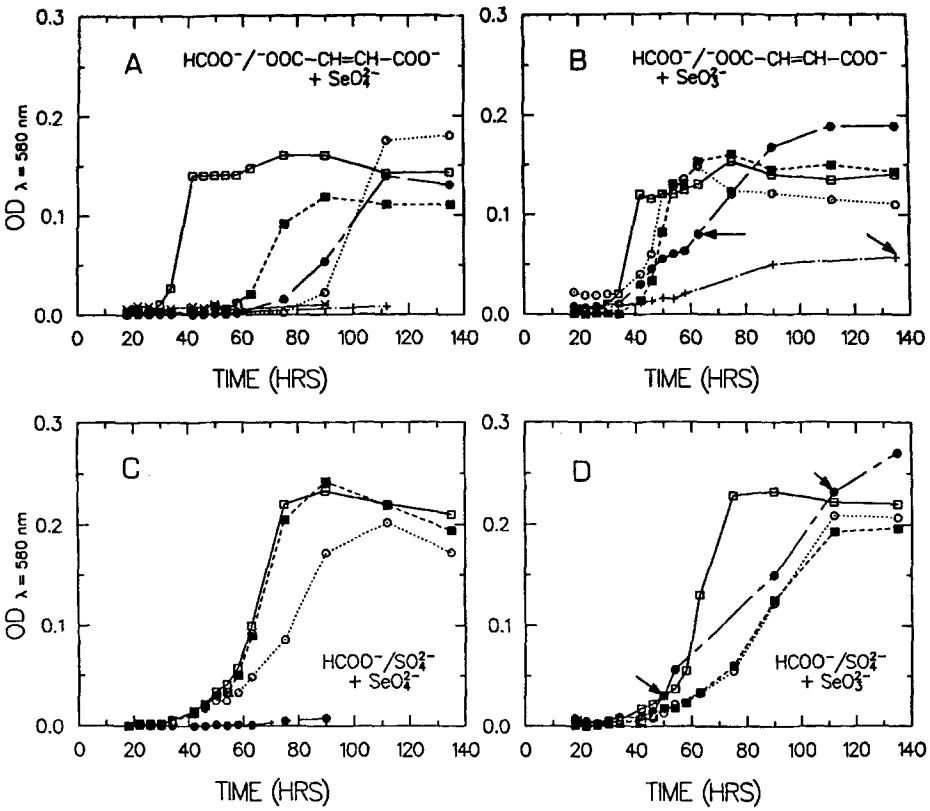

Fig. 1. Growth response with unadapted cultures of *D. desulfuricans*. (A) Formate/fumarate medium containing selenate. (B) As in (A) but the medium contained selenite. (C) Formate/sulfate medium containing selenate. (D) As in (C) but the medium contained selenite. Additions of selenate or selenite: none, open squares; 1 μM, solid squares; 10 μM, open circles; 100 μM, solid circles; 1 mM, plus signs; 10 mM, asterisks.

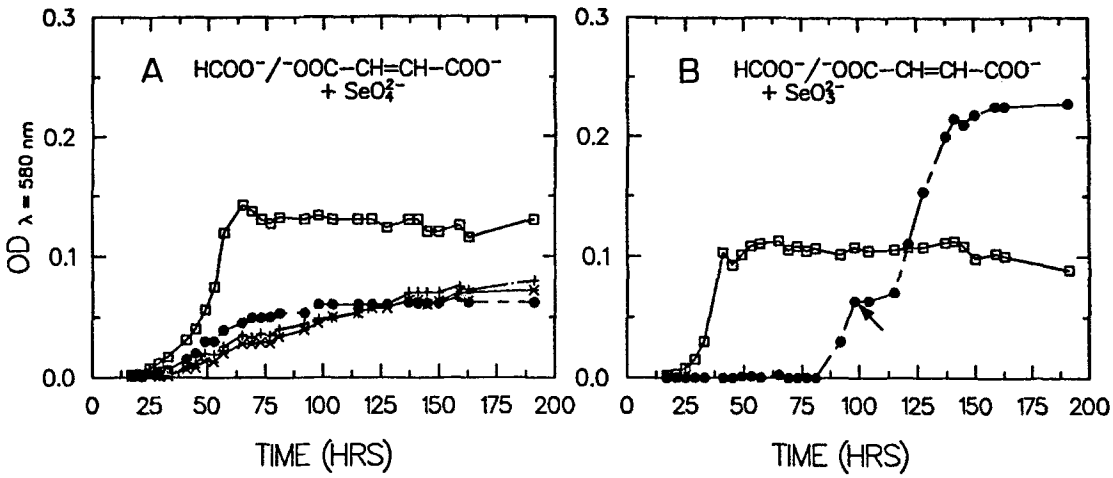

Fig. 2. Growth response of adapted cultures of *D. desulfuricans*. (A) Formate/fumarate medium containing selenate with inoculum from 100 μM selenate. (B) Formate/fumarate medium containing selenite with inoculum from 100 μM selenite. Additions of selenate or selenite: none, open squares; 100 μM, solid circles; 1 mM, plus signs; 10 mM, asterisks.

10 mM selenate at a rate comparable to cultures not receiving cysteine and elemental selenium was produced as the culture reached early stationary phase. Repeated transfer of bacteria in cysteine-supplemented media containing 0.1 mM selenite resulted in a growth rate that was similar to media not containing cysteine or selenite. Even with cysteine added to the media, *D. desulfuricans* did not grow in media containing 1 mM or 10 mM selenite.

It was necessary to adhere rigidly to a suitable scheme for the addition of selenium compounds and cysteine to avoid chemical reactions. If cysteine was added to the pH-equilibrated media before selenite, no elemental selenium was produced; however, if selenite was added before cysteine, red elemental selenium was instantly produced. With selenate there was no evidence of a chemical reaction with cysteine; therefore, the sequence for cysteine addition was less critical.

TABLE 1

Selenate and selenite reduced by *D. desulfuricans* with assays conducted after cells reached the stationary phase

Concentration added to medium	Selenate		Selenite	
	μg Se reduced	% Se reduced	μg Se reduced	% Se reduced
1 μM	0.7	95	0.7	94
10 μM	5.3	67	7.3	92
100 μM	18.0	23	76.3	97
1 mM	21.0	3	111.0	14
10 mM	7.0	<1	8.8	<1

Measurement of elemental selenium

Cultures that grew in the presence of selenate or selenite in formate/fumarate media were analyzed for elemental selenium after reaching stationary phase. Elemental selenium was produced at all concentrations of selenate and selenite (Table 1); however, red colloidal selenium could be detected only in cultures containing an initial concentration of <0.1 mM selenate or selenite. To visually detect a red color at 0.01 mM level of selenium oxyanions, it was necessary to allow the cells to settle to the bottom of the tubes. The small amount of elemental selenium produced at the 1 mM or 10 mM levels of selenium are a reflection of the poor growth that occurred in these unadapted cultures.

Electron microscopy

D. desulfuricans grown in the presence of selenite displayed altered form (Fig. 3) while cells grown in selenate-containing media had a curved cell similar to cells not exposed to selenium. Cells taken from selenate or selenite cultures at the time of early logarithmic phase did not show internal granules; however, cells taken from stationary phase cultures contained one to three large granules. The production of granules in selenate media appeared to be near the periphery of the cell (Fig. 4) while the granules were clearly intracytoplasmic and nonmembrane enveloped in cells grown in media containing selenite (Fig. 5). These granules were analyzed using energy-dispersive X-ray spectrometry and shown to contain selenium as the only detectable element heavier than sodium (Fig. 6). When this selenium granule was subjected to X-ray diffraction analysis, no regular diffraction pattern was displayed indicating that selenium in the granule was amorphous and not crystalline. A study by electron microscopy of stationary phase cultures revealed cell fragments and individual granules. This supported our hypothesis that red selenium colloid in the culture was a result of cell lysis with the liberation of internal selenium granules.

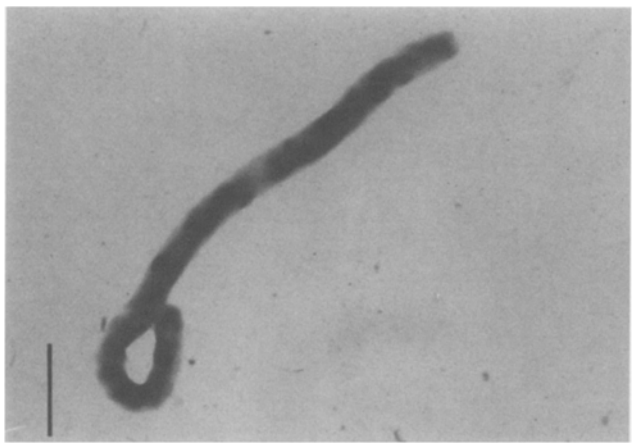

Fig. 3. Transmission electron microscopy of *D. desulfuricans* cells showing distorted cell morphology. Unstained cell taken from a culture growing in medium containing 100 μM selenite (marker = 0.5 μm).

Metabolic observations

Selenate and selenite did not have an effect on the metabolism of *D. desulfuricans*. From the analysis by ^{13}C nuclear magnetic resonance of carbon compounds in the medium following growth, the oxidation of formate with reduction of fumarate to succinate was observed. There was no evidence for fumarate dismutation when selenium oxyanions were present. The NMR spectrum shown in Fig. 7(A) is typical of the carbon compounds detected in uninoculated media. Fig. 7(B) is the spectrum of the culture tube following growth, indicating that succinate is the end product of fumarate reduction when

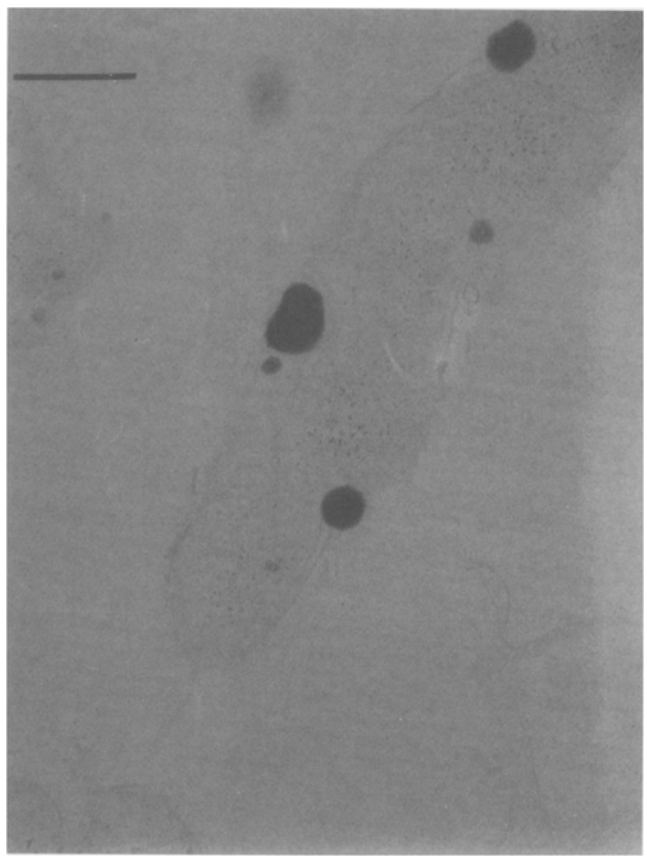

Fig. 4. Thin section of bacterial cell cultivated in formate/fumarate medium containing 100 μM selenate (marker = 0.2 μm).

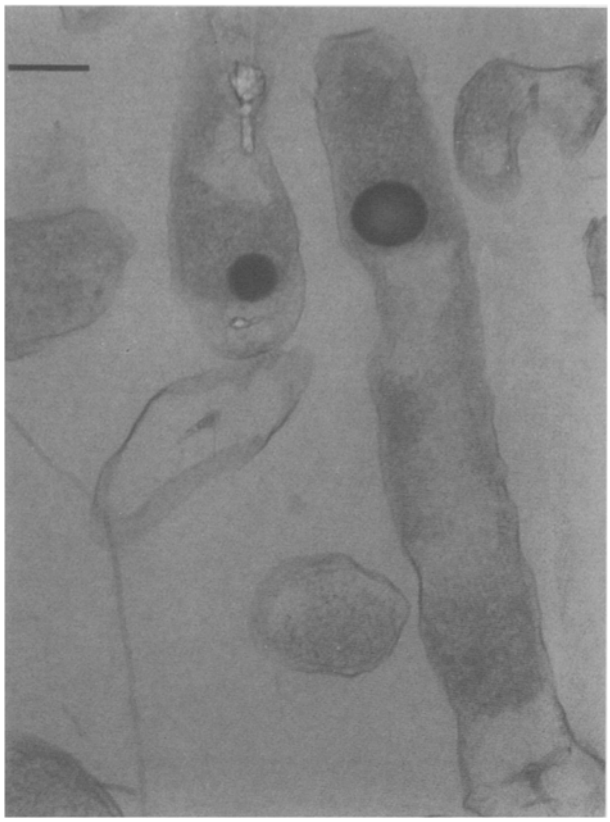

Fig. 5. Thin section of bacterial cell cultivated in formate/fumarate medium containing 100 μM selenite (marker = 0.25 μm).

no selenium is present. When selenate or selenite was added to this medium, NMR analysis of the media following growth indicates that succinate was the only product of metabolism (Fig. 7(C and D)). In selenium-free media where formate was not added, *D. desulfuricans* dismutated fumarate to acetate and succinate. When selenate or selenite was added to media where fumarate was both electron donor and electron acceptor, only acetate was produced (see Fig. 7(E)). If selenate or selenite would have promoted the fermentation of fumarate, ethanol would have been produced instead of acetate and this was not observed.

D. desulfuricans failed to grow or produce elemental selenium in fumarate-free media containing 0.1–10 mM selenate or selenite. Inocula used for these tests included cultures unadapted to selenium and cultures that grew in 100 μM selenate or 100 μM selenite.

We did not find evidence for the production of H_2Se, dimethylselenide, or dimethyldiselenide by *D. desulfuricans* growing in the presence of selenate or selenite. No ^{77}Se NMR signal for HSe^- or for selenite was detected in stationary phase cultures of *D. desulfuricans* grown in media containing 10 mM selenate. The sensitivity of this testing was sufficient to detect 100 μM selenide. Examination of numerous X-ray spectra from electron microscopy microprobe analysis indicated that there were no particles present in the bacteria or on the surface of the cell that contained both iron and selenium. If selenide had been produced in this medium with high iron content, ferrous selenide would have been produced. Finally, red elemental selenium was not produced when culture filtrates were exposed to the air for prolonged periods.

DISCUSSION

This is the first report to characterize the reduction of selenate or selenite to elemental selenium with internal deposition by a sulfate-reducing bacterium. This report establishes that

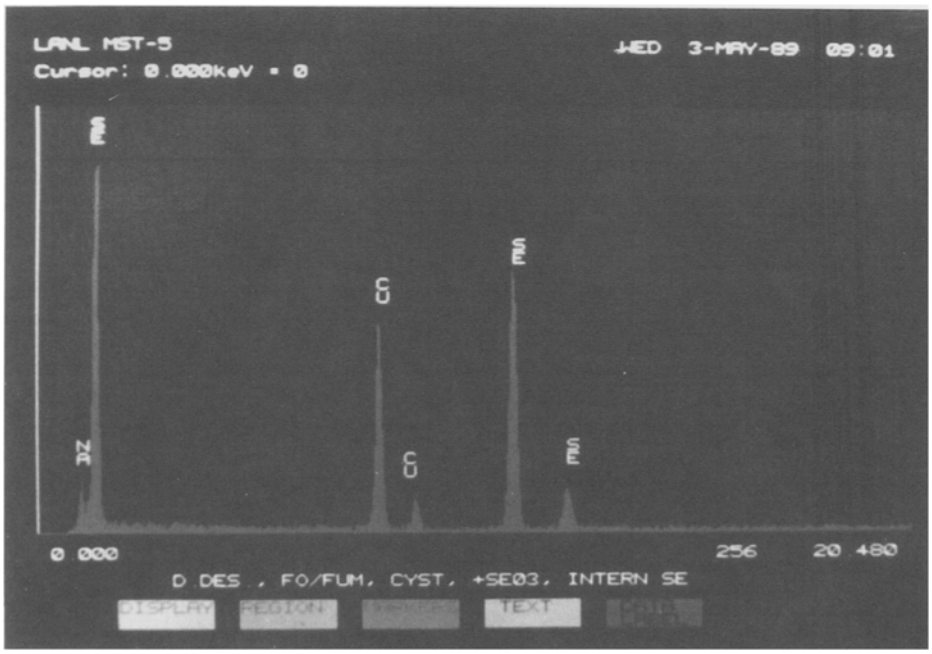

Fig. 6. Energy-dispersive X-ray spectrometry of internal granules. Cells grown in 100 μM selenite.

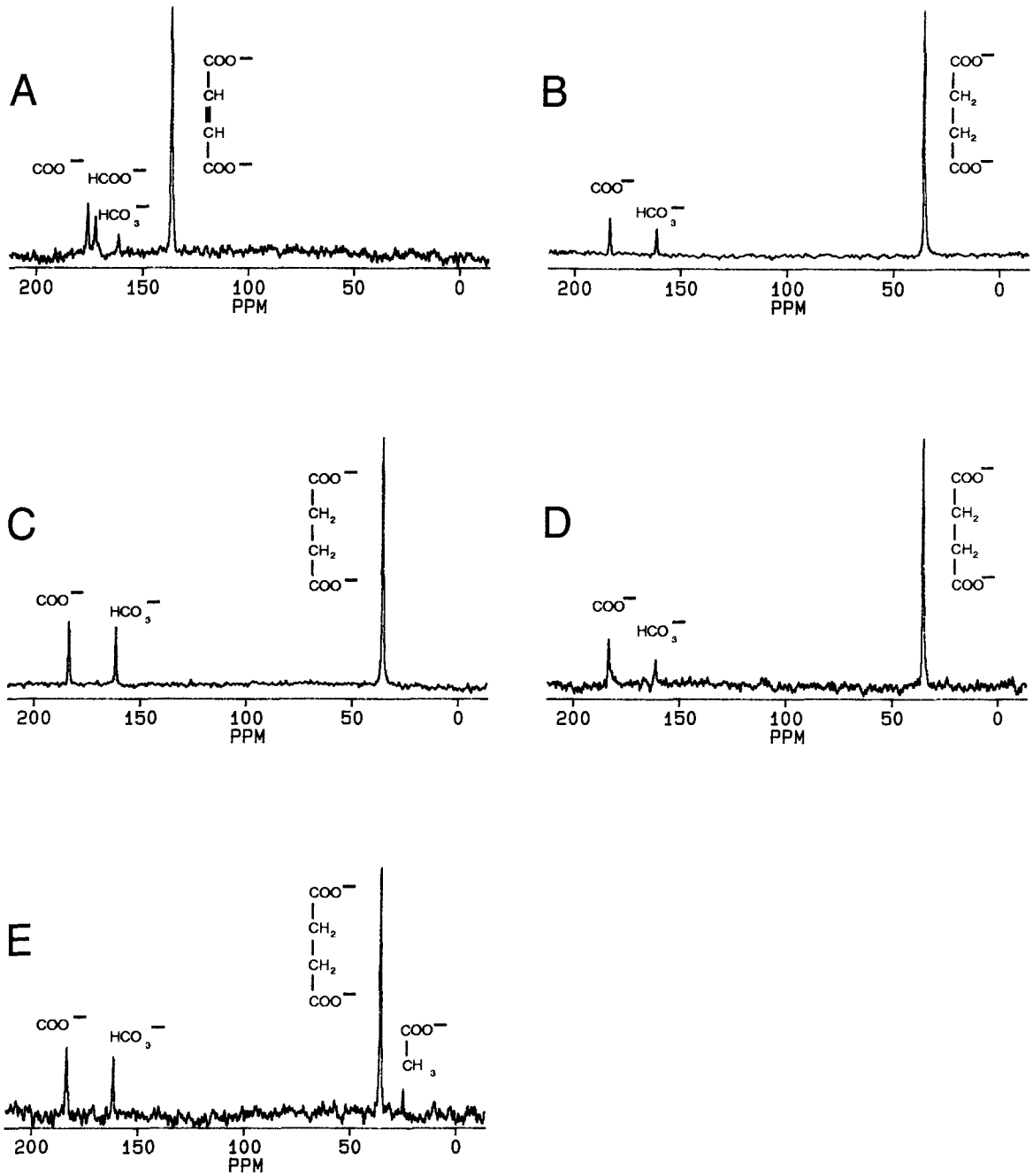

Fig. 7. Natural abundance (1%) ^{13}C nuclear magnetic resonance (NMR) spectra of carbon compounds in the growth medium. (A) Tube inoculated with *D. desulfuricans* containing 10 mM selenate prior to growth. (B) Tube inoculated with *D. desulfuricans* but no selenate or selenite present in the medium. Analysis conducted after culture has reached stationary phase. (C) and (D) as in (B) but the media contained 100 μM selenate or 100 μM selenite, respectively. (E) The tube was inoculated with *D. desulfuricans* into fumarate medium which did not contain formate. The medium contained 100 μM selenite. Identification of ^{13}C NMR peaks: 1, $-^{13}CH_2-^{13}CH_2-$ of succinate; 2, $-^{13}COO^-$ of succinate; 3, $H^{13}CH=^{13}CH-$ of fumarate; 5, $-^{13}COO^-$ of fumarate; 6, $H^{13}COO^-$; 7, $^{13}CH_3-$ of acetate.

reduction of selenate and selenite by *D. desulfuricans* is attributed to cytoplasmic activities and not due to a chemical reaction in the growth media. The development of tolerance to either of the two selenium species and their reduction to elemental selenium were found to be two separate phenomena. The visual appearance of red elemental selenium in the medium was preceeded by a cessation of growth. While the mechanisms of cell resistance to selenate and selenite remains unresolved in sulfate-reducing bacteria, characteristics demonstrated by these bacteria merit consideration.

Inhibition of growth for *D. desulfuricans* may be attributed to a structural similarity between selenate and sulfate. Selenate interferes with sulfate activation, the first step in the assimilatory or dissimilatory reduction of sulfate [1,10,36]. This results in the synthesis and subsequent hydrolysis of the adenosine-5′-phosphosulfate (APS) analog adenosine-5′-phosphoselenate

(APSe) to AMP and selenate [34,36]. Thus, selenate metabolism results in depleting the cell of ATP and reducing the rate of growth. Under the conditions where *D. desulfuricans* grows with fumarate instead of sulfate as the electron acceptor for growth, the requirement of sulfate is reduced and there is no competition between selenate and sulfate in dissimilatory reduction.

The pathway of assimilatory sulfate reduction has been shown to be highly regulated and the addition of cysteine to culture medium represses assimilatory sulfate reduction in bacteria [11,14,29]. With the addition of cysteine to the medium, the use of hydrogen sulfide to synthesize cysteine [17] is not required and assimilatory sulfate reduction would not function. The increased tolerance of *D. desulfuricans* to selenite or selenate in media containing cysteine may reflect the lack of sulfate utilized by these bacteria. Alternately, glutathione production may increase in the presence of cysteine and under conditions employed in this experiment glutathione production may occur. Selenite resistance has been attributed to glutathione production in *Escherichia coli* [31]; however, sulfate-reducing bacteria do not produce measurable levels of glutathione under standard growth conditions [12]. The formation of elemental selenium may follow the glutathione reaction scheme previously discussed [6]. Clearly the production of glutathione and glutathione reductase merits additional study in sulfate-reducing bacteria.

While several strains of *Desulfovibrio* can grow with the dismutation of fumarate [13], *D. desulfuricans* continued to dismutate fumarate with the production of succinate and acetate even when selenate or selenite were added to fumarate-containing media. Selenite in particular could have inhibited coenzyme A-mediated production of acetate from fumarate since selenite is known to react with thiol groups and participate in oxidation-reduction reactions with biological material [15,16,27]. This wide spectrum of toxicity mechanisms could explain the inability of *D. desulfuricans* to grow at levels of selenite above 0.1 mM. Additionally, one can infer that selenite concentration in the cytoplasm is below the level required to inhibit cellular metabolism. High concentrations of selenite in the cell wall region could interfere with thiol groups resulting in distorted cell morphology observed here (Fig. 3), with *W. succinogenes* [35] and with *Clostridium pasteurianum* [21].

In a related report by Zehr and Ormland [37], nanomolar levels of selenate and selenite were reduced to hydrogen selenide by *D. desulfuricans* subsp. *aestuarii* (ATCC 17990). The formation of hydrogen selenide from selenate by *D. desulfuricans* subsp. *aestuarii* was inhibited by sulfate with no hydrogen selenide produced from selenate when the sulfate concentration was greater than 10 mM. As reported here, the reduction of selenate to elemental selenium by *D. desulfuricans* subsp. *desulfuricans* occurred in the presence of 40 mM sulfate. Although the publication [37] did not mention that colloidal selenium was produced by *D. desulfuricans* (ATCC 17990), the production of elemental selenium from hydrogen sulfide reacting with selenate and selenite has been reported [30]. In future experiments, it would appear useful to examine environmental isolates to determine the frequency at which sulfate-reducing bacteria can produce elemental selenium in high sulfate-containing environments.

Clearly, the levels of selenium tolerated by *D. desulfuricans* would enable these organisms to participate in a bioremediation process. A characterization of selenium granules produced by bacteria revealed that surface charge on the granule is similar to that observed on bacterial cells [3,5]. Thus, fairly simple designs could be developed to decontaminate natural waters where selenium granules have been produced from sulfate-reducing bacteria [4].

ACKNOWLEDGEMENTS

This research was supported in part by the US Department of Energy. L.L.B. was the recipient of a grant from the Western Associated Universities and UNM Faculty Research Allocations. The authors thank Robert J. Sebring (Los Alamos National Laboratory) and Angela Welford (University of New Mexico) for contributions to the electron microscopy study. The authors recognize G. Salzman and R.G. Johnson (Los Alamos National Laboratory) for providing laboratory facilities.

REFERENCES

1 Akagi, J.M. and L.L. Campbell. 1962. Studies on thermophilic sulfate-reducing bacteria. III. Adenosine triphosphate-sulfurylase of *Clostridium nigrificans* and *Desulfovibrio desulfuricans*. J. Bacteriol. 84: 1194–1201.

2 Baldwin, R.A., J.C. Stauter, J.W. Kauffman, W.C. Laughlin. 1985. United States Patent number 4 519 913.

3 Barton, L.L., F.A. Fekete, E.V. Marietta, H.E. Nuttall, Jr and R. Jain. 1992. Potential for bacterial remediation of waste sites containing selenium or lead. In: Transport and Remediation of Subsurface Contaminants (D.A. Sabatini and R.C. Knox, eds), pp. 99–108, American Chemical Society, Washington, DC.

4 Barton, L.L., W.C. Lindemann and D.L. Bearden. 1992. Colloid formation as an approach to remediate toxic wastes containing chromium and lead. In: Waste Management 92, pp. 1305–1308, Arizona Board of Regents, Tucson.

5 Barton, L.L., H.E. Nuttall, W.C. Lindemann and R.C. Blake II. 1994. Biocolloid formation: an approach to bioremediation of toxic metal wastes. In: Remediation of Hazardous Waste Contaminated Soils (D.L. Wise and D.J. Trantolo, eds), pp. 481–496, Marcell Dekker, New York.

6 Blake, R.C. II, D. Cholate, S. Bardhan, N. Revis, L.L. Barton and T.G. Zocco. 1993. Chemical transformation of toxic metals by a *Pseudomonas* strain from a toxic waste site. Environ. Toxicol. Chem. 12: 1365–1376.

7 Bradbury, J.H. and G.A. Jenkins. 1984. Determination of the structures of trisaccharides by ^{13}C-N.M.R. spectroscopy. Carbohydrate Res. 126: 125–156.

8 Brown, T.A. and A. Shrift. 1980. Assimilation of selenate and selenite by *Salmonella typhimurium*. Can. J. Microbiol. 26: 671–675.

9 Bryant, R.D. and E.J. Laishley. 1988. Evidence for two transporters of sulfur and selenium oxyanions in *Clostridium pasteurianum*. Can. J. Microbiol. 34: 700–703.

10 Dilworth, G.L. and R.S. Bandurski. 1977. Activation of selenate by adenosine 5′-triphosphate sulfurylase from *Saccharomyces cerevisiae*. Biochem. J. 163: 521–529.

11 Dreyfuss, J. and K.J. Monty. 1963. Coincident repression of the reduction of 3′-phosphoadenosine 5′-phosphosulfate, sulfite, and thiosulfate in the cysteine pathway of *Salmonella typhimurium*. J. Biol. Chem. 238: 3781–3783.

12 Fahey, R.C., W.C. Brown, W.B. Adams and M.B. Worsham. 1978. Occurrence of glutathione in bacteria. J. Bacteriol. 133: 1126–1129.

13 Fauque, G., J. Le Gall and L.L. Barton. 1991. Sulfate-reducing and sulfur-reducing bacteria. In: Variations in Autotrophic Life (J.M. Shively and L.L. Barton, eds), pp. 271–339, Academic Press, London.

14 Fels, I.G. and V.H. Cheldelin. 1949. Selenate inhibition studies. III. The reversal of selenate inhibition in *E. coli*. Arch. Biochem. 22: 323–324.

15 Ganther, H.E. 1968. Selenotrisulfides: formation by the reaction of thiols with selenious acid. Biochem. 7: 2898–2905.

16 Ganther, H.E. and C. Corcoran. 1969. Selenotrisulfides. II. Cross-linking of reduced pancreatic ribonuclease with selenium. Biochem. 8: 2557–2563.

17 Gervitz, D., R. Amelunxen and J.M. Akagi. 1980. Cysteine synthesis by *Desulfovibrio vulgaris* extracts. J. Bacteriol. 141: 1460–1462.

18 Harrison, G.I., E.J. Laishley and H.R. Krouse. 1980. Stable isotope fractionation by *Clostridium pasteurianum*. 3. Effect of SeO_3^{2-} on the physiology and associated sulfur isotope fractionation during SO_3^{2-} and SO_4^{2-} reductions. Can. J. Microbiol. 26: 952–958.

19 Hudman, J.F. and A.R. Glenn. 1985. Selenium uptake by *Butyrivibrio fibrisolvens* and *Bacteroides ruminicola*. FEMS Microbiol. Lett. 27: 215–220.

20 Kemp, J.D., D.E. Atkinson, A. Ehret and R.A. Lazzarini. 1963. Evidence for the identity of the nicotinamide adenine dinucleotide phosphate-specific sulfite and nitrite reductases of *Escherichia coli*. J. Biol. Chem. 238: 3466–3471.

21 Laishley, E.J., G.I. Harrison, R.D. Bryant and H.R. Krouse. 1980. Influence of selenium compounds on reduction of sulfur compounds and associated sulfur isotope fractionation in *Clostridium pasteurianum*. In: Biochemistry of Ancient and Modern Sediments (P.A. Trudinger, M.R. Walter and B.J. Ralph, eds), pp. 261–266, Australian Acad. Sci., Canberra.

22 Lepage, S.P. and S. Bascomb. 1968. Use of selenite reduction in bacterial classification. J. Appl. Bacteriol. 31: 568–580.

23 Lindblow-Kull, C., F.J. Kull and A. Shrift. 1985. Single transporter for sulfate selenate and selenite in *Escherichia coli* K-12. J. Bacteriol. 163: 1267–1269.

24 Macy, J.M., T.A. Michel and D.G. Kirsch. 1989. Selenate respiration by a *Pseudomonas* species: a new mode of anaerobic respiration. FEMS Microbiol. Lett. 61: 195–198.

25 Newport, P.J. and D.B. Nedwell. 1988. The mechanism of inhibition of *Desulfovibrio* and *Desulfotomaculum* species by selenate and molybdate. J. Appl. Bacteriol. 65: 419–423.

26 Odom, J.D., W.H. Dawson and P.D. Ellis. 1979. Selenium-77 relaxation time studies on compounds of biological importance: dialkyl selenides, dialkyldiselenides, selenols, selenium compounds, and seleno oxyacids. J. Am. Chem. Soc. 101: 5815–5822.

27 Painter, E.P. 1941. The chemistry and toxicity of selenium compounds, with special reference to the selenium problem. Chem. Rev. 28: 179–213.

28 Pardee, A.B., L.S. Prestidge, M.B. Whipple and J. Dreyfuss. 1966. A binding site for sulfate and its relation to sulfate transport into *Salmonella typhimurium*. J. Biol. Chem. 241: 3962–3969.

29 Pasternak, C.A. 1962. Sulphate activation and its control in *Escherichia coli* and *Bacillus subtilis*. Biochem. J. 85: 44–49.

30 Postgate, J. 1952. Competitive and non-competitive inhibitors of bacterial sulphate reduction. J. Gen. Microbiol. 6: 128–142.

31 Schmidt, M.G. and W.A. Konetzka. 1986. Glutathione overproduction by selenite-resistant *Escherichia coli*. Can. J. Microbiol. 32: 825–827.

32 Spallholz, J.E., G.F. Collins and K. Schwartz. 1978. A single test tube method for the microdetermination of selenium. Bioinorg. Chem. 9: 453–459.

33 Spurr, A.R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26: 31–43.

34 Taylor, B.F. and R.S. Oremland. 1979. Depletion of adenosine triphosphate in *Desulfovibrio* by oxyanions of group VI elements. Curr. Microbiol. 3: 101–103.

35 Tomei, F.A., L.L. Barton, C.L. Lemanski and T.G. Zocco. 1992. Reduction of selenate and selenite to elemental selenium by *Wolinella succinogenes*. Can. J. Microbiol. 38: 1328–1333.

36 Wilson, L.G. and R. Bandurski. 1958. Enzymatic reactions involving sulfate, sulfite, selenate, and molybdate. J. Biol. Chem. 233: 975–981.

37 Zehr, J.P. and R.S. Oremland. 1987. Reduction of selenate to selenide by sulfate-respiring bacteria: experiments with cell suspensions and estuary sediments. Appl. Environ. Microbiol. 53: 1365–1369.