
Biogeochemistry **55:** 73–86, 2001.

Biotransformations of selenium by *Enterobacter cloacae* SLD1a-1: Formation of dimethylselenide

ROBERT S. DUNGAN & WILLIAM T. FRANKENBERGER, JR*
*Department of Environmental Sciences, University of California, Riverside CA 92521, U.S.A. (*Author for correspondence, Fax: +1 (909) 787-2954, e-mail: william.frankenberger@ucr.edu)*

Key words: biomethylation, biotransformations, dimethylselenide, demethylselenone, selenium

Abstract. Whole-cell suspensions of *Enterobacter cloacae* SLD1a-1 produced dimethylselenide (DMSe) from selenate, selenite, elemental selenium, dimethylselenone, seleno-DL-methionine, 6-selenoinosine, and 6-selenopurine. Cell-free extracts of the bacterium produced the formation of DMSe from organic selenium compounds, including dimethylselenone, dimethylselenoniopropionate, seleno-DL-methionine, seleno-DL-ethionine, and 6-selenoguanosine. The highest rate of DMSe production occurred from whole-cell suspensions and cell-free extracts containing dimethylselenone. DMSe was also produced by cell-free extracts containing selenite or elemental selenium and methylcobalamin. Cell-free extracts did not produce DMSe from inorganic selenium when *S*-adenosyl-L-methionine was present. Additionally, DL-homocysteine and L-methionine were found to inhibit selenium volatilization. These findings suggest the formation of DMSe from inorganic selenium occurs through the transfer of a methyl group from methylcobalamin.

Introduction

Selenium (Se) is of particular interest because it is both required by and toxic to animals (Frankenberger & Enberg 1998). In the environment, it is well known that the biological cycling of Se is very similar to that of sulfur. Microorganisms, which play important roles in the cycling of Se, transform Se through oxidation, reduction, methylation, and demethylation reactions. As a result, Se occurs in a variety of mineralized, water soluble, or gaseous forms, which subsequently alter its bioavailability and behavior in the environment.

The biological transformation of nonvolatile Se to volatile compounds is a major pathway in the global flux of Se from terrestrial and marine environments to the atmosphere (Haygarth 1994). The methylation of Se in seleniferous environments is readily carried out by fungi and bacteria, and

is thought to be a detoxification mechanism. Dimethylselenide (DMSe) is the major volatile gas produced by most Se methylating bacteria and fungi, although lesser amounts of other volatile compounds such as dimethyldiselenide (DMDSe), dimethyl selenenyl sulfide (DMSeS), and methane selenol (CH_3SeH) may also be produced (Dungan & Frankenberger 1999). The biomethylation of Se is of particular interest because it represents a potential technology to detoxify Se-contaminated soils, sediments, and waters.

Since Challenger (1945) proposed a pathway on the microbial methylation of Se, a number of other Se methylation pathways have been proposed (Reamer & Zoller 1980; Doran 1982; Cook & Bruland 1987). In general, the formation of DMSe from selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) occurs through successive reduction and methylation steps. Reported methyl donors in the microbial methylation of inorganic Se are methylcobalamin and *S*-adenosylmethionine (McBride & Wolfe 1971; Doran & Alexander 1977). Proposed organic intermediates in the formation of DMSe include dimethylselenone [$(CH_3)_2SeO_2$] (Challenger 1945; Reamer & Zoller 1980; Zhang & Chasteen 1994), selenomethionine (Cook & Bruland 1987), and dimethylselenoniopropionate (DMSeP) (Ansede & Yoch 1997). Doran (1982) proposed that SeO_3^{2-} is reduced via elemental Se (Se^0) to a selenide form before it is methylated to form methane selenol and finally DMSe. Although some knowledge has been gained in the area of microbial Se methylation, little progress has been made in validating these proposed pathways.

Enterobacter cloacae SLD1a-1, a facultative anaerobe known to reduce SeO_4^{2-} and SeO_3^{2-} to Se^0 (Losi & Frankenberger 1997; Dungan & Frankenberger 1998), was found to volatilize Se in the presence of SeO_3^{2-} (Dungan & Frankenberger 2000). The volatile gas was identified as DMSe. To our knowledge, this is the first time that an enteric bacterium has been reported to be a methylator of Se. The intent of this study was to characterize *E. cloacae* SLD1a-1 as a model organism to provide additional information on the Se methylation pathway.

Materials and methods

Chemicals. Tryptic Soy Broth (TSB) and Tryptic Soy Agar (TSA) were obtained from Difco (Detroit, MI). Sodium selenate (Na_2SeO_4), sodium selenite (Na_2SeO_3) and acrylic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). DMSe was obtained from Strem Chemical Co. (Newburyport, MA). Seleno-DL-methionine, seleno-DL-cystine, seleno-DL-ethionine, 6-selenoinosine, 6-selenopurine, 6-selenoguanosine,

cyanocobalamin (vitamin B_{12}), methylcobalamin, betaine, choline chloride, DL-5-methyl-tetrahydrofolic acid, *S*-adenosyl-L-methionine, DL-homocysteine, and 3-chloroperoxybenzoic acid were purchased from Sigma Chemical Co. (St. Louis, MO). L-Methionine and L-ascorbic acid were obtained from Fisher Scientific (Fair Lawn, NJ). Sodium selenide (Na_2Se) was obtained from Alfa Aesar (Ward Hill, MA).

$(CH_3)_2SeO_2$ was prepared by the method of Zhang and Chasteen (1994). DMSeP was synthesized using the procedure of Ansede and Yoch (1997). Se^0 (red colloidal) was prepared by dissolving approximately 1 g of Na_2SeO_3 in 10 mL of deionized water and then adding excess L-ascorbic acid. Red Se^0 was immediately visible upon the addition of L-ascorbic acid. The mixture was shaken on an orbital shaker (150 rpm) at room temperature for 15 min. The precipitate was then removed by vacuum filtration. The procedure was repeated several times with the filtrate until no more Se^0 could be obtained. The crude Se^0 was then washed three times in deionized water and collected by centrifugation (12,000 $\times$ g for 10 min). The final product was then air-dried and pulverized into a fine powder.

Growth conditions. Enterobacter cloacae SLD1a-1 was routinely maintained on TSA medium (40 g L^{-1}). For whole-cell suspension and cell-free extract experiments the organism was grown aerobically in 2 L batches of TSB (30 g L^{-1}) at 28 °C with shaking (120 rpm). The starter culture (100 mL of TSB) was inoculated with a loop of colony from an agar plate and prepared under the same conditions. After 18 h, the cells were harvested by centrifugation (6,000 $\times$ g, 10 min, 5 °C) and washed three times in cold 50 mM potassium phosphate buffer (pH 6.9).

Whole-cell suspensions. To determine the ability of *E. cloacae* SLD1a-1 to transform various inorganic and organic Se compounds into DMSe, whole-cell suspensions were amended with either SeO_4^{2-}, SeO_3^{2-}, Se^0, Na_2Se, seleno-DL-methionine, seleno-DL-cystine, seleno-DL-ethionine, 6-selenoguanosine, 6-selenoinosine, 6-selenopurine, $(CH_3)_2SeO_2$ or DMSeP. The whole-cell suspensions were prepared by resuspending the cells in cold 50 mM phosphate buffer such that a 10-fold dilution gave an optical density of 0.4 at 600 nm. 25-mL aliquots of the whole-cell suspension were dispensed into 40-mL vials, amended with an Se compound (0.1 mM, except Se^0 and Na_2Se, which were tested at 100 mM) and glucose (20 mM), and capped with Teflon-lined septa. The vials were then incubated at 28 °C for 24 h on an orbital shaker (120 rpm). Heat-killed controls were prepared by autoclaving an aliquot of the cell suspension (120 °C, 18 psi, 20 min) and then treating it exactly as the live cell suspension. After 24 h, the headspace

was analyzed for DMSe by gas chromatography. Each treatment was carried out in triplicate.

Cell-free extracts. Cell-free extracts were prepared by resuspending the cells in 40 mL of cold 50 mM phosphate buffer and sonically disrupting them with four 1-min bursts (Fisher Scientific, model 550 Sonic Dismembrator, Pittsburgh, PA). The cell suspension was cooled in an ice bath between sonic bursts. The extracts were clarified of cellular debris by centrifugation (12,000 $\times$ g, 10 min, 5 °C). The protein concentration in the cell-free extracts was determined by the Bradford (1976) method, using bovine serum albumin as the standard.

To determine if inorganic Se could be transformed into DMSe by cell-free extracts, 1 mL of extract was amended with either 0.1 mM of SeO_3^{2-} or 100 mM of Se^0, 0.05 mM of $MgCl_2$, and 1 mg of one of the following methyl donors: *S*-adenosyl-L-methionine, betaine, choline, cyanocobalamin, methylcobalamin, L-methionine, or DL-5-methyl tetrahydrofolic acid. The protein concentration of the extract was 3.5 mg mL^{-1}. Control reactions contained no methyl donor.

To assess the ability of cell-free extracts to form DMSe from organic Se, seleno-DL-methionine, 6-selenoinosine, 6-selenopurine, seleno-DL-cystine, seleno-DL-ethionine, 6-selenoguanosine, $(CH_3)_2SeO_2$, or DMSeP was added to 1 mL of extract (5.3 mg protein mL^{-1}) containing no $MgCl_2$. The concentration of all organo-Se substrates was 0.1 mM. Controls contained autoclaved (120 °C, 18 psi, 20 min) cell-free extracts that were treated exactly as non-autoclaved extracts.

Each of the reactions was carried out in an 8.5-mL vial capped with Teflon-lined septa. The vials were incubated on an orbital shaker (120 rpm) at 28 °C and the headspace was sampled periodically for DMSe.

Gas chromatography. Using a gas-tight syringe (Pressure-Lok, Baton Rouge, LA), a 1-mL headspace sample was withdrawn from the whole-cell suspensions or cell-free extracts and analyzed for DMSe by injecting it directly into a GC. The syringes were cleaned by heating them to 55 °C between sample injections. A blank injection was performed prior to each sample injection to assure the syringes were free of volatile Se. The GC analysis was performed on a Hewlett-Packard (Avondale, PA) model 5890 GC equipped with a flame-ionization detector. The detector signal was processed by a Hewlett-Packard 3396A Series II integrator. The operational conditions were as follows: stainless steel column (10 m long and 2.2 mm I.D.); liquid phase, 10% Carbowax 1000; solid support, Chrom W-AW; particle size, 0.18 to 0.24 mm (mesh 60/80); column temperature, 65 °C;

injector and detector temperature, 105 °C; carrier gas, He, 30 mL min^{-1}; H_2, 33 mL min^{-1}; air, 320 mL min^{-1}. Quantification and identification of DMSe was achieved by using an authentic standard.

Effect of DL-*homocysteine and* L-*methionine.* The effect of DL-homocysteine and L-methionine on the volatilization of Se was evaluated by growing *E. cloacae* SLD1a-1 in a liquid medium of TSB (pH 7.3) containing 0.01 mM SeO_3^{2-} and DL-homocysteine or L-methionine at the following concentrations: 0.01, 0.1, 1.0, and 10 mM. The TSB was added in 100-mL quantities to 250-mL Erlenmeyer flasks and autoclaved (20 min, 121 °C, 18 psi) before the addition of SeO_3^{2-} and DL-homocysteine or L-methionine. To quantify the volatile Se released from the microcosms, 3.7×10^3 Bq of the radiotracer ^{75}Se (as Na_2SeO_3) was added to each flask. All reagents were prepared in deionized water and filter-sterilized (0.2 μm) prior to their addition to the medium. To initiate the experiment, each flask was inoculated with 1 mL of a 24-h culture of *E. cloacae* SLD1a-1. The inoculum was prepared in TSB and incubated at 22 °C on an orbital shaker (150 rpm). The microcosms were incubated at 22 °C under static conditions. Each treatment consisted of three replicates.

To sample for volatile Se, moistened air was flushed through two-hole stoppered 250-mL Erlenmeyer flasks at a flow rate of approximately 100 mL min^{-1}. The outlet air from each flask was passed through two stacked activated carbon cartridges (14 mm o.d., 55 mm long, containing approximately 1.8 g of activated carbon) to trap the volatile Se. Activated C traps were collected approximately every 24 h and samples which could not be analyzed on the same day were frozen at −20 °C until the analysis could be performed. The second cartridge was used to detect breakthrough and was analyzed separately. Each of the activated C traps were analyzed directly for ^{75}Se without any further preparation. All activated C samples were measured for ^{75}Se using a gamma counter (Norland 5000 MultiChannel Analyzer System, Fort Atkinson, WI) with a NaI crystal (Bicron, Newbury, OH).

Results and discussion

Whole-cell suspensions. The ability of a whole-cell suspension of *E. cloacae* SLD1a-1 to produce DMSe from inorganic and organic Se compounds is shown in Table 1. The following Se compounds were not transformed to DMSe: Na_2Se, seleno-DL-cystine, seleno-DL-ethionine, and 6-selenoguanosine. No DMSe was produced in the reaction vials when heat-killed cells were used, except when DMSeP and $(CH_3)_2SeO_2$ were the substrates. Heat-killed cells containing DMSeP produced twice as much

Table 1. Production of DMSe by whole-cell suspensions of *Enterobacter cloacae* SLD1a-1.*

Se compound	DMSe (ng mL^{-1} headspace)
Selenate	9.4 (2.8**)
Selenite	2.5 (1.2)
Elemental Se	10.8 (0.1)
Selenomethionine	9.6 (2.4)
Selenoinosine	2.3 (0.3)
6-Selenopurine	2.7 (0.9)
Dimethylselenoniopropionate	11.8 (0.3)
Dimethylselenone	790.1 (12.8)

*Whole-cell suspensions were incubated at 28 °C for 24 h. The concentration of all Se compounds was 0.1 mM, except elemental Se at 100 mM.
**Values in parentheses represent the standard deviation of three replicates.

DMSe as the live cells (data not shown), indicating that the reaction was largely abiotic in nature. Apparently, DMSeP is not stable under the conditions provided and is readily degraded to DMSe through chemical mechanisms. As a result, no determination can be made to the amount of DMSe, if any, that was biologically produced from DMSeP. Live suspensions amended with $(CH_3)_2SeO_2$ reached maximum DMSe production after 1 h and no DMSe was produced from heat-killed cells for up to 7 h (data not shown). Of the inorganic Se compounds (i.e., SeO_4^{2-} and SeO_3^{2-}), *E. cloacae* SLD1a-1 produced 3.8 times more DMSe from SeO_4^{2-} as from SeO_3^{2-}. *Alternaria alternata*, a fungal isolate, produced approximately 1.3 times more DMSe from SeO_4^{2-} than from SeO_3^{2-} (Thompson-Eagle et al. 1989). However, biomethylation rates from SeO_3^{2-} amended bacterial and fungal suspensions were generally higher when compared to SeO_4^{2-} (Barkes & Fleming 1974; Doran & Alexander 1977). Since Se oxyanions must first be reduced before they can be methylated, Se volatilization rates from SeO_4^{2-} are generally lower than SeO_3^{2-} because of its higher redox potential. *Enterobacter cloacae* SLD1a-1 may have produced less DMSe in the presence of SeO_3^{2-} because it is generally considered more biologically toxic than SeO_4^{2-} (Oehme 1972). Apparently high SeO_3^{2-} concentrations are also toxic to *E. cloacae* SLD1a-1, since 11.3 times more DMSe was produced at a SeO_3^{2-} concentration of 0.01 mM than at 1.0 mM (Dungan & Frankenberger 2000). Selenite is a powerful oxidizing agent, which readily denatures sulfhydryl enzymes and oxidizes sulfhydryl groups to disulfides and selenotrisulfides (Doran 1982).

With respect to Se^0, whole-cell suspensions of *E. cloacae* SLD1a-1 produced 4.3 times more DMSe than from SeO_3^{2-} and slightly more than from SeO_4^{2-} (Table 1). In contrast, Doran and Alexander (1977) found that a *Corynebacterium* sp. methylated Se^0 approximately 3.4 and 1.8 times less than SeO_4^{2-} and SeO_3^{2-}, respectively. Reamer and Zoller (1980) found that DMSe was generated more slowly from soil and sewage sludge amended with Se^0 than with SeO_3^{2-}. The low solubility of Se^0 is often considered a contributing factor to its reduced biomethylation when compared to the highly soluble SeO_4^{2-} and SeO_3^{2-} ions. However, under the conditions provided, whole-cell suspensions of *E. cloacae* SLD1a-1 were equally or better able to produce DMSe from Se^0 than from the Se oxyanions. These results are as expected if one considers the potential energy savings by utilizing reduced forms of Se, such as Se^0. The ability of *E. cloacae* SLD1a-1 to produce DMSe from SeO_4^{2-}, SeO_3^{2-}, and Se^0 supports Doran's (1982) hypothesis that inorganic Se may be transformed to DMSe via the intermediate Se^0. However, it was postulated that Se^0 was reduced to an unknown selenide form before methylation occurred. Although Na_2Se was tested as an intermediate in this study, the inability of *E. cloacae* SLD1a-1 to produce DMSe from Na_2Se could be due to its bioavailability, as a result of its very low solubility or the selenide form used. In addition, it cannot be ruled out that a selenide intermediate may not exist.

Whole-cell suspensions of *E. cloacae* SLD1a-1 also transformed organic forms of Se, including $(CH_3)_2SeO_2$, seleno-DL-methionine, 6-selenoinosine, and 6-selenopurine into DMSe (Table 1). The production of DMSe from cell suspensions containing seleno-DL-methionine and 6-selenoinosine and 6-selenopurine was approximately equivalent to that of SeO_4^{2-} and SeO_3^{2-}, respectively. The largest amount of DMSe was produced when $(CH_3)_2SeO_2$ was used as the substrate. Figure 1 shows the transformation of $(CH_3)_2SeO_2$ to DMSe by *E. cloacae* SLD1a-1 over a 2.5-h incubation period. Challenger (1945) proposed that $(CH_3)_2SeO_2$ is the last intermediate prior to the formation of DMSe in *Scopulariopsis brevicaulis*. Unfortunately, Challenger never identified $(CH_3)_2SeO_2$ in the medium and this has not been accomplished to date. Zhang and Chasteen (1994) reported that *Pseudomonas fluorescens* K27 produced significantly more DMSe in the presence of $(CH_3)_2SeO_2$ than in the presence of either SeO_4^{2-} or SeO_3^{2-}. The formation of DMSe by *E. cloacae* SLD1a-1 could indicate that $(CH_3)_2SeO_2$ is an intermediate in the formation of DMSe as postulated by Challenger. Alternatively, it may be equally possible that *E. cloacae* SLD1a-1 derives benefit from degrading $(CH_3)_2SeO_2$ to DMSe.

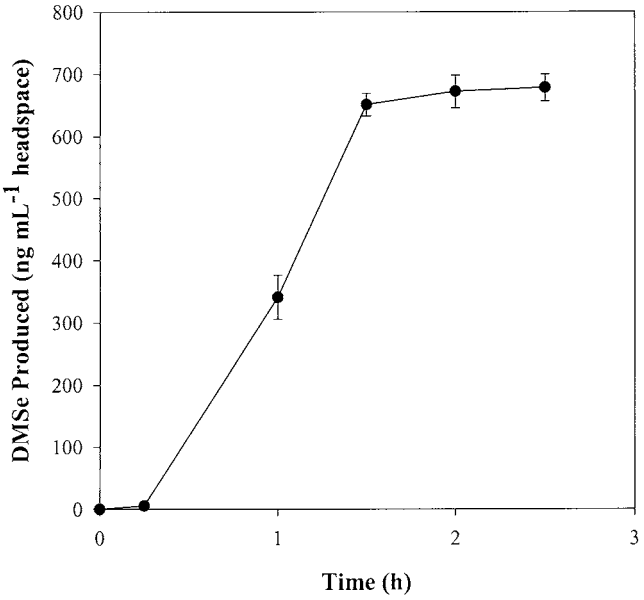

Figure 1. Evolution of DMSe by whole-cell suspensions of *E. cloacae* SLD1a-1 containing 0.1 mM dimethylselenone. The cell suspensions were incubated on an orbital shaker (120 rpm) at 28 °C. Data points are the average of triplicate samples and error bars represent the standard deviation.

Cell-free extracts. Several compounds are known to donate methyl groups in various methylation reactions, including *S*-adenosylmethionine, tetrahydrofolate, betaine, and methylcobalamin (Stryer 1995). Of these methyl donors, only *S*-adenosylmethionine and methylcobalamin are known to donate methyl groups during the biomethylation of inorganic Se. Doran and Alexander (1977) identified a *Corynebacterium* sp. which utilized *S*-adenosylmethionine as a cofactor in the formation of DMSe. In mammals, *S*-adenosylmethionine is required for Se methylation (Tandon et al. 1986; Hoffman & McConnell 1987). Cell-free extracts of *E. cloacae* SLD1a-1, amended with either SeO_3^{2-} or Se^0, were able to catalyze the formation of DMSe in the presence of methylcobalamin (Figure 2), but not *S*-adenosyl-L-methionine (data not shown). Although Se methylation occurred via methylcobalamin (analog of vitamin B_{12}), cell-free extracts did not produce DMSe when vitamin B_{12} (cyanocobalamin) was tested as the methyl donor. Dimethylselenide production was the highest from cell-free extracts containing SeO_3^{2-}, with 76 ng of DMSe produced per mg of protein in 3 h. The volatilization of DMSe was roughly linear when Se^0 was the substrate and after 4 h, approximately 9.5 times less DMSe was produced when compared with that of SeO_3^{2-}. Once again, the reduced methyla-

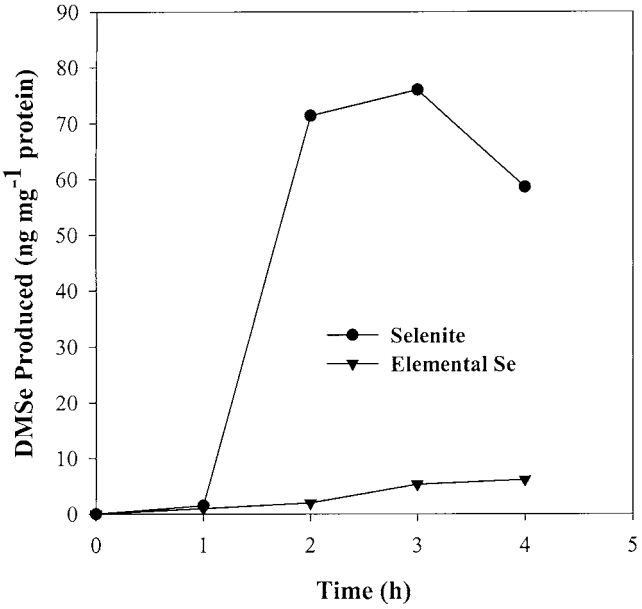

Figure 2. Formation of DMSe by cell-free extracts of *E. cloacae* SLD1a-1 containing selenite (0.1 mM) or elemental Se (100 mM) and methylcobalamin (1 mg mL^{-1} extract) as the methyl donor. Each reaction vial contained 1 mL of protein extract (3.5 mg protein mL^{-1}) and 0.05 mM $MgCl_2$ and was incubated at 28 °C.

tion of Se^0 may be due to its very low solubility. No DMSe formation occurred in cell-free extracts containing SeO_3^{2-} or Se^0 when methylcobalamin was absent (data not shown). Methylcobalamin has been isolated from bacteria (Linstrand 1964). McBride and Wolfe (1971) found that cell-free extracts of a *Methanobacterium* sp. methylated SeO_4^{2-}, arsenate (AsO_4^{3-}), and tellurate (TeO_4^{2-}) when methylcobalamin was present. Methylcobalamin is also involved in the biomethylation of several other metals, including mercury, tin, palladium, and platinum (Ridley et al. 1977). Choi et al. (1994) proposed that the synthesis of methylmercury by *D. desulfuricans* LS occurred via methylcobalamin. Thompson-Eagle et al. (1989) found the addition of methylcobalamin promoted the methylation of SeO_4^{2-} by *A. alternata*, with an 8.6-fold increase in DMSe production over the control. Addition of betaine, choline, L-methionine, and DL-5-methyl tetrahydrofolic acid to cell-free extracts of *E. cloacae* SLD1a-1, containing either SeO_3^{2-} or Se^0, did not produce DMSe. According to Challenger's Se methylation pathway, betaine, choline, and methionine could act as methyl donors for Se.

To assess the ability of cell-free extracts to enzymatically catalyze the formation of DMSe from organic Se compounds, each of the various Se

compounds previously tested were added to the cell-free extracts. The cell-free extracts catalyzed the formation of DMSe from $(CH_3)_2SeO_2$, DMSeP, seleno-DL-methionine, seleno-DL-ethionine, and 6-selenoguanosine. After 1 h of incubation, the extract produced 1.5, 0.7, and 0.8 ng of DMSe per mL of headspace when seleno-DL-methionine, seleno-DL-ethionine, and 6-selenoguanosine were the Se substrate, respectively. However, after two hours, DMSe was no longer detected in the reaction vial headspace. No DMSe was produced by autoclaved aliquots of the cell-free extract. In contrast to the whole-cell suspension experiment, no DMSe was formed by the extracts when 6-selenoinosine or 6-selenopurine was tested.

Figure 3 shows the production of DMSe, over a 4-h incubation period, by cell-free extracts containing $(CH_3)_2SeO_2$ or DMSeP. The production of DMSe was 120-fold greater from extracts containing $(CH_3)_2SeO_2$. Production of DMSe by cell-free extracts containing $(CH_3)_2SeO_2$, in addition to whole-cells, further supports $(CH_3)_2SeO_2$ as a precursor in the formation of DMSe. DMSeP has been detected in salt marsh cordgrass (*Spartina alterniflora*) and it is thought that it may serve as a precursor in the enzymatic formation of DMSe (Ansede et al. 1999). Many species of marine plankton contain dimethylsulfoniopropionate (DMSP), which is the major precursor of dimethylsulfide (DMS) and possibly the sulfur analog of DMSeP (Saltzman & Cooper 1989). Two isolates, *Alcaligenes* sp. strain M3A and *Pseudomonas doudoroffii*, when induced by DMSP, produced DMSe when DMSeP was the substrate (Ansede & Yoch 1997). The enzymatic formation of DMSe from DMSeP by *E. cloacae* SLD1a-1, may be analogous to the aerobic degradation of DMSP by marine bacteria, which results in the production of DMS (Taylor & Gilchrist 1991). Although cell-free extracts containing DMSeP, catalyzed the formation of small amounts of DMSe, our results are tenuous at best, and do not exclude or imply that DMSeP is a precursor of DMSe.

Effect of DL-homocysteine and L-methionine. It is well known that methylcobalamin mediates the transfer of a methyl group to homocysteine to generate methionine during the activated methyl cycle (Stryer 1995). In *E. coli*, methylcobalamin was found to donate a methyl group to homocysteine, resulting in the formation of methionine (Foster et al. 1964). Since the methylation of inorganic Se by *E. cloacae* SLD1a-1 occurred when methylcobalamin was present, it is possible that Se is methylated during the generation of methionine. In the fungus *Neurospora crassa*, the formation of methyl mercury was inhibited by the addition of methionine (Lander 1971). It was speculated that mercury methylation by *N. crassa* was a side reaction of methionine biosynthesis. In a strain of *Penicillium*, the formation

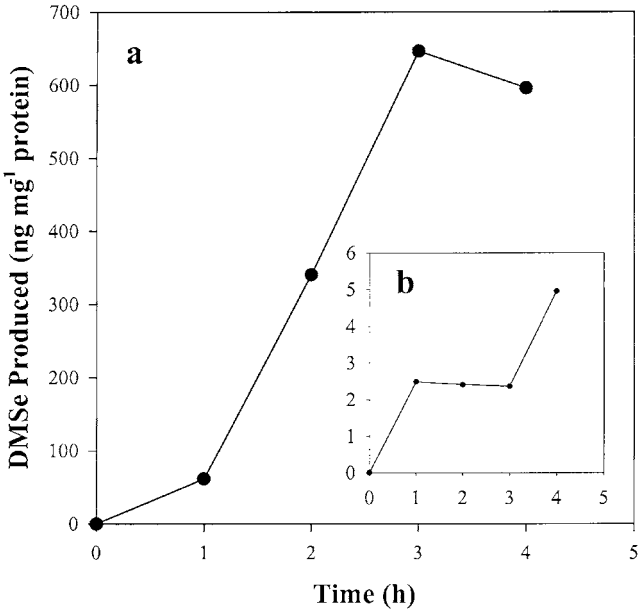

Figure 3. The transformation of organo-Se compounds (0.1 mM), dimethylselenone (a) and DMSeP (b), into DMSe by cell-free extracts of *E. cloacae* SLD1a-1. The extracts were incubated at 28 °C and the protein concentration was 5.3 mg mL^{-1}.

of DMSe from SeO_4^{2-} was also suppressed by methionine (Fleming & Alexander 1972). Figures 4(a) and 4(b) show that both L-methionine and DL-homocysteine inhibit the volatilization of Se by *E. cloacae* SLD1a-1. Based on these results, the formation of DMSe by *E. cloacae* SLD1a-1 could be a side reaction of methionine synthesis. However, this is largely circumstantial evidence, and additional research will be necessary to explore this possibility.

Selenium methylation pathways. Based on the information obtained in this study, the methylation of inorganic Se oxyanions by *E. cloacae* SLD1a-1 could be occurring through several possible pathways as postulated by Challenger (1945), Doran (1982), and Cook and Bruland (1987). Dimethyl selenomethionine (Cook & Bruland 1987), methane selenol (Doran 1982), and methane selenonic acid and methane seleninic acid (Challenger 1945), which have been suggested as intermediates in the methylation of inorganic Se, were not tested in this study. The rapid production of DMSe in the presence of $(CH_3)_2SeO_2$ implies that $(CH_3)_2SeO_2$ is a potential precursor in the formation of DMSe, possibly the last intermediate prior to the formation of DMSe as postulated by Challenger. Additionally, organic intermediates other than those tested in this study may exist since seleno-DL-ethionine, 6-

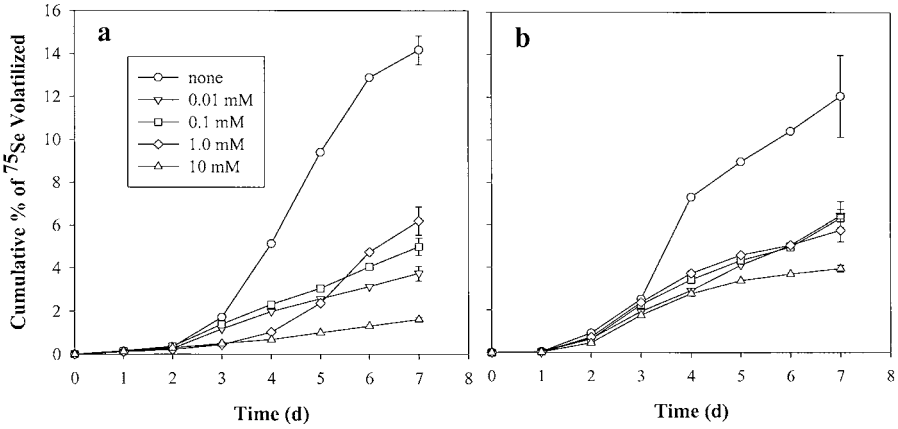

Figure 4. The influence of DL-homocysteine (a) and L-methionine (b) on the volatilization of Se by *E. cloacae* SLD1a-1. The bacterium was grown in a liquid medium of TSB and incubated statically at 22 °C. Each treatment contained 0.01 mM SeO_3^{2-}.

selenoinosine, 6-selenopurine, and 6-selenoguanosine were transformed to DMSe. However, it should be noted that the ability of *E. cloacae* SLD1a-1 to produce DMSe from the inorganic and organic Se compounds tested in this study is not definitive proof of their existence as intermediates, nor is it proof of a mechanism. Further investigation will be required to determine if these Se compounds are indeed intermediates in the Se methylation pathway. As of now, there is no clear understanding as to why *E. cloacae* SLD1a-1 methylates and volatilizes Se. A detoxification mechanism seems unlikely because DMSe production occurs in nutrient-rich and -poor medium at low Se concentrations.

References

Ansede JH & Yoch DC (1997) Comparison of selenium and sulfur volatilization by dimethylsulfoniopropionate (DMSP) lyase in two marine bacteria and estuarine sediments. FEMS Microbiol. Ecol. 23: 315–324

Ansede JH, Pellechia PJ & Yoch DC (1999) Selenium biotransformation by the salt marsh cordgrass *Spartina alterniflora*: Evidence for dimethylselenoniopropionate formation. Environ. Sci. Technol. 33: 2064–2069

Barkes L & Fleming RW (1974) Production of dimethylselenide gas from inorganic selenium by eleven soil fungi. Bull. Environ. Contamin. Toxicol. 12: 308–311

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal. Biochem. 72: 248–254

Challenger F (1945) Biological methylation. Chem. Rev. 36: 315–361

Choi S-C, Chase T & Bartha R (1994) Metabolic pathways leading to mercury methylation in *Desulfovibrio desulfuricans* LS. Appl. Environ. Microbiol. 60: 4072–4077

Cook TD & Bruland KW (1987) Aquatic chemistry of selenium: Evidence of biomethylation. Environ. Sci. Technol. 21: 1214–1219

Doran JW & Alexander M (1977) Microbial transformations of selenium. Appl. Environ. Microbiol. 33: 31–37

Doran JW (1982) Microorganisms and the biological cycling of selenium. In: Marshall KC (Ed.) Advances in Microbial Ecology (pp 1–32). Plenum Press, New York, NY

Dungan RS & Frankenberger Jr WT (1998) The reduction of selenite to elemental selenium by *Enterobacter cloacae* SLD1a-1. J. Environ. Qual. 27: 1301–1306

Dungan RS & Frankenberger Jr WT (1999) Microbial transformations of selenium and the bioremediation of seleniferous environments. Bioremediation J. 3: 171–188

Dungan RS & Frankenberger Jr WT (2000) Factors affecting the volatilization of dimethylselenide by *Enterobacter cloacae* SLD1a-1. Soil Biol. Biochem. 32: 1353–1358

Fleming RW & Alexander M (1972) Dimethylselenide and dimethyltelluride formation by a strain of *Penicillium*. Appl. Microbiol. 24: 424–429

Foster MA, Dilworth MJ & Woods DD (1964) Cobalamin and the synthesis of methionine by *Escherichia coli*. Nat. (London) 201: 39–42

Haygarth PM (1994) Global importance of global cycling of selenium. In: Frankenberger Jr WT & Benson S (Eds.) Selenium in the Environment (pp 1–28). Marcel Dekker, Inc., New York, NY

Hoffman JL & McConnell KP (1987) Periodate-oxidized adenosine inhibits the formation of dimethylselenide and trimethylselenonium ion in mice treated with selenite. Arch. Biochem. Biophys. 254: 534–540

Lander L (1971) Biochemical model for the biological methylation of mercury suggested from methylation studies in vivo with *Neurospora crassa*. Nat. 230: 452–454

Linstrand K (1964) Isolation of methylcobalamin from natural source material. Nat. 204: 188–189

Losi ME & Frankenberger Jr WT (1997) Reduction of selenium oxyanions by *Enterobacter cloacae* strain SLD1a-1: Isolation and growth of the bacterium and its expulsion of selenium particles. Appl. Environ. Microbiol. 63: 3079–3084

McBride BC & Wolfe RS (1971) Biosynthesis of dimethylarsine by methanobacterium. Biochem. 10: 4312–4317

Oehme FW (1972) Mechanisms of heavy metal toxicities. Clin. Toxicol. 5: 131–167

Oldfield JE (1998) Environmental implications of uses of selenium with animals. In: Frankenberger Jr WT & Enberg RA (Eds.) Environmental Chemistry of Selenium (pp 129–142). Marcel Dekker, Inc., New York, NY

Reamer DC & Zoller WH (1980) Selenium biomethylation products form soil and sewage sludge. Sci. 208: 500–502

Ridley WP, Dizikes LJ & Wood JM (1977) Biomethylation of toxic elements in the environment. Sci. 197: 329–332

Saltzman ES & Cooper WJ (1989) Biogenic sulfur in the environment. Am. Chem. Soc. Symp. Series No. 393. Washington, DC

Stryer L (1995) Biochemistry, 4th edn. W.H. Freeman and Company, New York

Tandon SK, Magos L & Webb M (1986) The stimulation and inhibition of the exhalation of volatile selenium. Biochem. Pharmacol. 35: 2763–2766

Taylor BF & Gilchrist DC (1991) New routes for aerobic biodegradation of dimethylsulfoniopropionate. Appl. Environ. Microbiol. 57: 3581–3584

Thompson-Eagle ET, Frankenberger Jr WT & Karlson U (1989) Volatilization of selenium by *Alternaria alternata*. Appl. Environ. Microbiol. 55: 1406–1413

Zhang L & Chasteen TG (1994) Amending cultures of selenium-resistant bacteria with dimethyl selenone. Appl. Organometal. Chem. 8: 501–508.