

Arsenic and Selenium in Microbial Metabolism*

John F. Stolz,¹ Partha Basu,² Joanne M. Santini,³ and Ronald S. Oremland⁴

¹Department of Biological Sciences, Duquesne University, Pittsburgh, Pennsylvania 15282; email: stolz@duq.edu

²Department of Chemistry and Biochemistry, Duquesne University, Pittsburgh, Pennsylvania 15282; email: basu@duq.edu

³Department of Biology, University College London, London WC1E 6BT, United Kingdom; email: j.santini@ucl.ac.uk

⁴Water Resources Division, U.S. Geological Survey, Menlo Park, California 94025; email: roremlan@usgs.gov

Annu. Rev. Microbiol. 2006. 60:107–30

First published online as a Review in Advance on May 8, 2006

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi:
10.1146/annurev.micro.60.080805.142053

Copyright © 2006 by Annual Reviews.
All rights reserved

0066-4227/06/1013-0107\$20.00

*The U.S. Government has the right to retain a nonexclusive, royalty-free license in and to any copyright covering this paper.

Key Words

selenate respiration, selenocysteine, arsenate reductase, arsenite oxidase, biogeochemical cycles, organoarsenicals

Abstract

Arsenic and selenium are readily metabolized by prokaryotes, participating in a full range of metabolic functions including assimilation, methylation, detoxification, and anaerobic respiration. Arsenic speciation and mobility is affected by microbes through oxidation/reduction reactions as part of resistance and respiratory processes. A robust arsenic cycle has been demonstrated in diverse environments. Respiratory arsenate reductases, arsenic methyltransferases, and new components in arsenic resistance have been recently described. The requirement for selenium stems primarily from its incorporation into selenocysteine and its function in selenoenzymes. Selenium oxyanions can serve as an electron acceptor in anaerobic respiration, forming distinct nanoparticles of elemental selenium that may be enriched in ⁷⁶Se. The biogenesis of selenoproteins has been elucidated, and selenium methyltransferases and a respiratory selenate reductase have also been described. This review highlights recent advances in ecology, biochemistry, and molecular biology and provides a prelude to the impact of genomics studies.

Contents

INTRODUCTION.....	108
Phylogeny and Diversity.....	108
Ecology.....	109
ARSENIC IN CELLULAR	
METABOLISM.....	112
Methylation.....	112
Organoarsenicals.....	114
Arsenic Resistance.....	114
Respiratory Arsenate Reductase ...	115
Arsenite Oxidase.....	117
SELENIUM IN CELLULAR	
METABOLISM.....	118
Selenocysteine.....	119
Methylation.....	120
Respiratory Selenate Reductase ...	121
CONCLUSIONS AND FUTURE	
DIRECTIONS.....	121

INTRODUCTION

Selenium (Se) has been called an “essential toxin,” as it is required for certain cell processes and enzymes, but it becomes deleterious at greater doses. The requirement for selenium stems primarily from its incorporation into selenocysteine and its function in selenoenzymes. Arsenic (As) is also considered an essential toxin, but its beneficial qualities are more subtle. In humans, acute and chronic exposures result in defined pathologies (e.g., arsenicosis, arseniasis, and death), and yet arsenic can promote angiogenesis and increased respiratory capacity. In prokaryotes, these two elements are readily metabolized and participate in a full range of metabolic functions including assimilation, methylation, detoxification, and anaerobic respiration. Recent reviews have provided overviews of the microbial ecology of selenium (133) and arsenic (97, 98); the resistance mechanisms for arsenic (82, 112); and the biochemistry and molecular biology involved in selenoproteins (24), selenate respiration (123), and arsenic metabolism (15, 123, 131). This review high-

lights some of the most recent advances in ecology, biochemistry, and molecular biology and discusses the impact genomics studies are beginning to have.

Phylogeny and Diversity

Concerted efforts have been made over the past decade to isolate and characterize organisms capable of generating energy from oxidation/reduction reactions with oxyanions of arsenic and selenium. These efforts have been aided by the identification of environments where these elements play an important role in ecology (see below), the development of selective media, and the inclusion of arsenate and selenate as electron acceptors tested in the characterization of new isolates. As a result, the list of species capable of carrying out oxidation/reduction reactions with oxyanions of selenium and arsenic continues to grow.

Arsenite-oxidizing bacteria have been known since 1918 (34); however, the first chemolithoautotrophic species were described only recently (92, 121). More than 30 strains representing at least nine genera of arsenite-oxidizing prokaryotes have been reported and include α -, β -, and γ -Proteobacteria, and *Thermus* (97). Physiologically diverse, they include heterotrophic and chemolithoautotrophic arsenite-oxidizing prokaryotes (32, 117, 121, 122). It is also apparent that green sulfur (e.g., *Chlorobium limicola* and *Chlorobium phaeobacteroides*) and filamentous green nonsulfur bacteria (e.g., *Chloroflexus aurantiacus*) may be capable of arsenite oxidation, as homologs of arsenite oxidase have been identified in their genomes. Arsenite oxidation by Archaea has yet to be definitively demonstrated; however, arsenic oxidation and reduction in *Sulfolobus acidocaldarius* have been reported (130) and homologs of arsenite oxidase have been identified in the genomes of *Sulfolobus tokodaii* and *Aeropyrum pernix* (64). In addition, archaeal 16S rDNA sequences, those of both Crenarchaeota and Euryarchaeota,

Selenate respiration: the generation of energy through the dissimilatory reduction of selenate

Chemolithoautotrophic arsenite-oxidizing prokaryotes: microbes that generate energy through arsenite oxidation while using carbon dioxide for cell carbon

Heterotrophic arsenite-oxidizing prokaryotes: microbes that oxidize arsenite but require an alternative source of energy and organic matter for growth

were found to coincide with the zone of arsenite oxidation in a Yellowstone hot spring (50). Most species are aerobic, using oxygen as the electron acceptor. However, the γ -Proteobacterium *Alcalilimmicola ebrlichei* (strain MLHE-1) oxidizes arsenite under anoxic conditions while respiring nitrate (92), and two recently described chemolithoautotrophs, α -Proteobacterium strain DAO10 and β -Proteobacterium strain DAO1, couple arsenite oxidation to denitrification (110). Thus, arsenite can serve as an electron donor in anaerobic respiration. The discovery of arsenite oxidase in anaerobic photosynthetic bacteria poses the intriguing possibility that arsenite might be a source of electrons in photosynthesis.

Dissimilatory arsenate-reducing bacteria are a more recent discovery (3, 63, 70, 71, 87). Nevertheless, at last count at least 24 prokaryotes use arsenate as a terminal electron acceptor (97, 98). They include members of the Crenarchaeota, Aquificae, Chrysiogenes, Deferribacteres, low G+C gram-positive bacteria, Halanaerobacter, and γ -, δ -, and ϵ -Proteobacteria (98). A full range of electron donors are also employed including organics, such as acetate, lactate, and aromatics (e.g., syringic acid, ferulic acid, phenol, benzoate, toluene) (3, 51, 66, 86, 89, 134, 139), and inorganics (e.g., hydrogen, sulfide). The recently described chemolithoautotrophic arsenate respirer, strain MLMS-1 (a δ -Proteobacterium), couples the oxidation of hydrogen sulfide to arsenate reduction (43). Strain SLAS-1, a haloalkaliphilic extremophile isolated from Searles Lake, California, can use either lactate or sulfide as electron donors for growth on arsenate (94). Arsenate respiration is a variable trait within any taxonomic group. For example, six of the seven known species of *Sulfurospirillum* can respire arsenate (*S. barnesii*, *S. arsenophilum*, *S. deleyianum*, *S. multivorans*, *S. halorespirans*, *S. carboxydovorans*) (51, 68). With the possible exception of the chemolithoautotrophic strain MLMS-1, arsenate respiration is not obligate, as many species are capable of us-

ing other terminal electron acceptors such as oxygen, nitrate, nitrite, Fe(III), fumarate, sulfate, thiosulfate, sulfur, dimethyl sulfoxide, and trimethyl amine oxide (97).

Prokaryotes capable of gaining energy from oxidation/reduction reactions with oxyanions of selenium are also phylogenetically diverse and metabolically versatile. At present, 20 species are known and include members of the Crenarchaeota, low and high G+C gram-positive bacteria, Halanaerobacter, and β -, γ -, and ϵ -Proteobacteria. Many of the organisms also use arsenate as a terminal electron acceptor; however, only the haloalkaliphilic *Bacillus selenitireducens* (137) and three strains of an *Aquificales* species (HG MK-1, 2, and 3) (138) use selenite.

Ecology

Biogeochemical cycle of arsenic. Arsenic primarily occurs in four oxidation states, arsenate As(V), arsenite As(III), elemental As(0), and arsenide As(-III). Inorganic As(V) and As(III) are readily soluble with H_2AsO_4^- and HAsO_4^- occurring primarily in aerobic environments, and H_3AsO_3^0 and H_2AsO_3^- more common in anoxic environments. Elemental arsenic occurs rarely, and arsines have been identified from fungal cultures and strongly reducing environments (6). The importance of arsenic in microbial ecology and its biogeochemical cycle have also been realized only recently (97, 99, 94, 109). The activities of arsenic-metabolizing microbes can affect the speciation and mobility of arsenic. Arsenate-respiring bacteria can liberate arsenic [As(III)] from sediments (2, 54), from adsorptive sites of aluminum oxides or ferrihydrite (146), or from arsenate-containing minerals (i.e., scorodite) (86). Recent studies in Bangladesh have implicated microbial processes as a key contributor to arsenic contamination in near- and sub-surface aquifers (37, 48, 88, 97, 98).

In Mono Lake, California, arsenate respiration has been linked to the mineralization of 8% to 14% of the photosynthate fixed

Arsenate respiration: the generation of energy through the dissimilatory reduction of arsenate

during meromixis (46, 90). The highest rates of arsenate reduction were measured at the base of the oxycline ($5.5 \mu\text{mol l}^{-1}\text{d}^{-1}$), although activity could be measured throughout the anoxic bottom waters (90). The high rates of activity could not be supported solely by the influx of inorganic arsenate, suggesting arsenite oxidation. Furthermore, arsenate reduction appeared to be inhibited by nitrate (44). It was discovered later that nitrate reduction was coupled to arsenite oxidation and that a complete oxidation/reduction cycle for arsenic was operable in the metalimnion and hypolimnion (92, 99). This led to the isolation of the arsenite-oxidizing/nitrate-reducing bacterium *Alcalilimnicola ebrlichei* strain MLHE-1 (92). The subsequent isolation of the two denitrifying bacteria from arsenate-contaminated soils (strains DAO1 and DAO10) suggests that nitrate-linked anaerobic oxidation of arsenite is widespread in nature (110). More recently, arsenic cycling has been shown to drive the microbial ecology in Searles Lake (94). There, the saturated salt brines and high concentrations of arsenic ($\sim 3.9 \text{ mM}$) create an environment where sulfate reduction and methanogenesis are highly constrained, if not totally inhibited (94). Arsenate respiration is the dominant process in the anaerobic zone while arsenite oxidation predominates in the aerobic zone (94).

Carbon mineralization is not the only important function of these organisms.

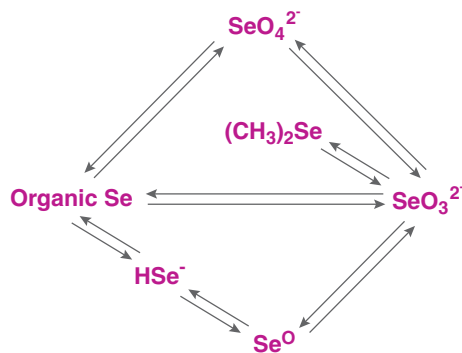


Figure 1

The selenium cycle.

Inorganic carbon fixation via chemolithoautotrophy has been demonstrated in several bacterial arsenite oxidizers (e.g., *Alcalilimnicola ebrlichei*, gold mine strain NT 26, strains DAO1 and DAO10) and in at least three bacterial species of arsenate respirers that can use either sulfide [i.e., Mono Lake strain MLMS-1 (43), Searles Lake strain SLAS-1 (94)] or hydrogen [*Desulfosporosinus* sp. strain Y5, (66)] as electron donors. Hydrogen has also been cited as an electron donor that supports chemoautotrophic growth of two species of arsenate-respiring Crenarchaea (47). Thus, these diverse arsenic-metabolizing microorganisms could contribute to the overall carbon budget of microbial communities in arsenic-rich environments by contributing to either the formation or the oxidation of the organic carbon found therein (43, 44, 90, 99, 121, 122, 124).

Biogeochemical cycle of selenium. Selenium occurs in four oxidation states, selenate Se(VI), selenite Se(IV), elemental selenium Se(0), and selenide Se(-II) (**Figure 1**). Se(-II) is found as organoselenium typically in the form of proteins containing the amino acids selenocysteine and selenomethionine or as metal selenide mineral phases in rocks and sediments (135). The common methylated species are volatile and contain Se(-II) (e.g., dimethyl selenide, dimethyl diselenide). The distribution of the different species may vary with the environment, but typically soluble selenate and selenite are found in the oxic zone and the insoluble Se(0) is more abundant in the anoxic zone (135). Hattori and colleagues (38) found that selenate was the dominant form in the surface waters of the Indian Ocean, with selenite concentration increasing with depth. Cutter & Bruland (18) found, however, that approximately 80% of the selenium in surface waters of the North and South Pacific Oceans was in the form of organoselenium. Its distribution was correlated with the maxima for primary productivity, pigment, and dissolved free amino acids. In water column profiles of well-stratified

marine environments, such as Saanich Inlet of British Columbia (17) and the Orca Basin in the Gulf of Mexico (57), the speciation of selenium changes from oxidized forms (e.g., selenate and selenite) to more reduced forms (organoselenium and hydrogen selenide) with transit from the oxic surface waters to the anoxic bottom waters. However, “organo-selenium” in these investigations is an operationally defined term and can potentially also include elemental selenium as well as selenium that is truly associated with proteins. Microbial selenate reduction has been linked directly to the production of Se(0) in a number of anoxic sediments (93, 95, 96). Thus, the common link is that selenium speciation in nature is strongly dependent on microbial activity (22).

The first mention of an organism capable of generating energy from selenium was a short note by Lipman and Waxman (65) published in *Science* in 1923. The promised follow-up publication with the description of the organism, however, never materialized. The oxidation of selenium to selenite by a heterotrophic organism, *Bacillus megaterium*, was described in 1981 (126). More recently, Dowdle & Oremland (22) established that *Lep-totrich* sp. strain MNB-1 and *Thiobacillus* sp. strain ASN-1 were capable of oxidizing selenium to selenate. However, these bacterial reactions as well as those of soils were very slow (i.e., rate constants = $0.5\text{--}1.0^{-y}$) and were not linked to energy conservation for the microbes involved.

Over the past two decades, the finer aspects of a selenium biochemical cycle have begun to emerge. The dissimilatory reduction of Se(VI) via Se(IV) to Se(0) has been shown to be a significant and rapid environmental process. While Se(IV) reduction to Se(0) also occurs in nature, few selenite-respiring bacteria are known (137, 138). Selenium reduction to selenide (HSe^{-1}) has only recently been described (42). The discovery of selenium isotope fractionation ($^{80}\text{Se}/^{76}\text{Se}$ of -8.0%) during selenite respiration by *B. selenitireducens*,

combined with the overall reduction of Se(VI) to Se(0) by *Bacillus arseniciselenatis* (-11.0%) and *Sulfurospirillum barnesii* (-12.4%) (40), presents the possibility that this novel respiratory pathway leaves a discernable biosignature in the rock record. However, chemical isotope fractionation by naturally occurring reductants (e.g., $\text{Fe}^{\text{II}}/\text{Fe}^{\text{III}}$ -containing “green rusts”) can also reduce selenium oxyanions to their elemental state with roughly the same discrimination factors as those observed for the microbiological reactions (53). This makes attribution to chemical or biological causative reactions of deposited selenium in sediments difficult, if not impossible, to achieve. Moreover, because relatively little $^{80}\text{Se}/^{76}\text{Se}$ variation (e.g., $0.6\text{--}2.0\%$) has actually been observed within natural ecosystems themselves, the utility of this approach to track in situ microbiological processes is questionable (41, 52).

Studies on microbial selenium methylation and demethylation are also few in number, the former process being studied as a means to remove selenium from contaminated agricultural soils (25, 29, 77, 106–108). Molecular analysis of freshwater environments suggested that selenium methylation activity was carried out by species of γ -Proteobacteria [e.g., *Pseudomonas* spp. (27)]. Demethylation of dimethyl selenide in anoxic sediments is carried out by methylotrophic methanogens via pathways established for growth on the analogous compound dimethyl sulfide (58, 100).

The formation of elemental selenium “nanospheres” by selenium-respiring bacteria is a phenomenon that deserves special mention. These exospheres are composed of native selenium and have roughly the same, uniform diameter ($0.2\text{--}0.3\ \mu\text{m}$). They occur outside the cell envelope and eventually slough off the cell surface and into the medium. When harvested and cleansed of their cellular parents, they were found to have curious electro-optical properties, making them candidates for further studies with “nanotechnological” applications (91).

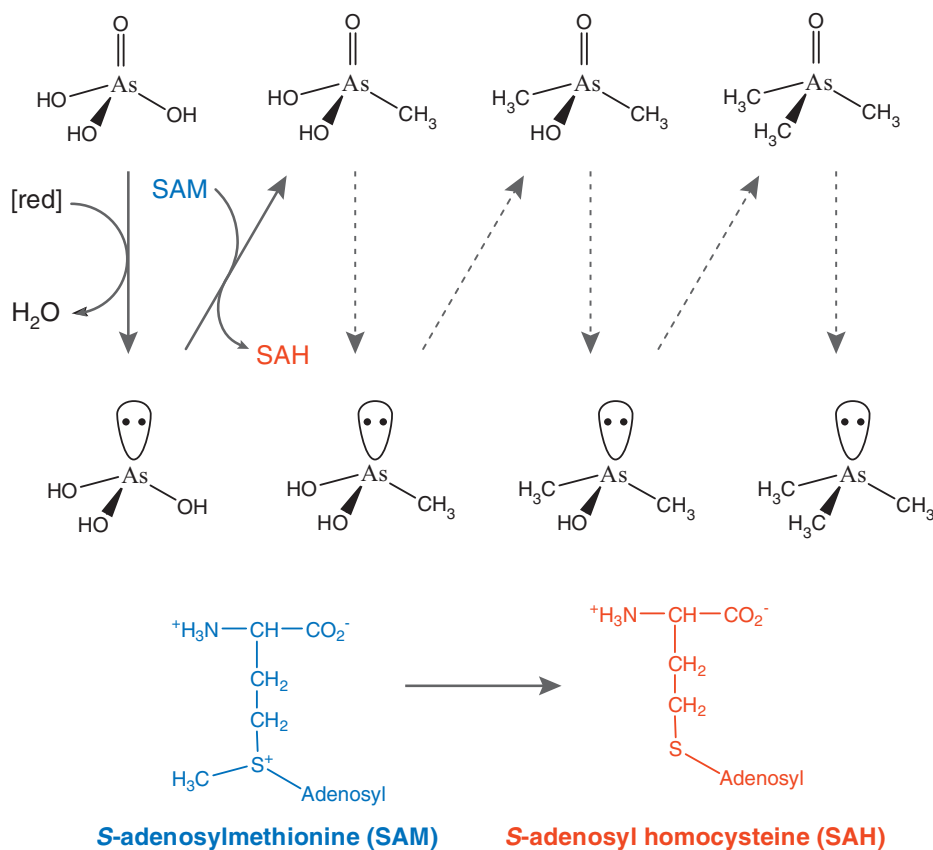
ARSENIC IN CELLULAR METABOLISM

Arsenic, despite its toxicity, is readily used by a great diversity of prokaryotes for cell growth and metabolism. While detoxification and energy generation are the primary processes, the discovery that organoarsenicals such as arsenobetaine and arsenolipids are involved in cellular functions suggests that some organisms may readily assimilate arsenic. Four basic processes of microbial arsenic transformation, methylation, demethylation, oxidation, and reduction, are discussed here.

Methylation

Methylation is thought to be a detoxification process; however, a growing body of literature suggests that not all methylated prod-

ucts are any less toxic than inorganic forms of arsenic. Although arsenic methylation by fungi and other eukaryotes has been well documented (as reviewed in References 29 and 6), less is known for bacterial systems. The pathway, as originally proposed by Challenger (12), based on work with the fungus *Scopulariopsis brevicaulis*, involves a series of steps in which the reduction of arsenate is followed by oxidative addition of a methyl group (12, 21). This addition results in the formation of different compounds including methyl arsenite, dimethyl arsenate, dimethyl arsenite, and trimethyl arsine oxide (TMA₂SO) (Figure 2). The same pathway has also been suggested for prokaryotes, although the formation of arsine (Me_xAsH_{3-x}, x = 0–2) is a feature more common in bacteria (6). The volatilization of arsenicals was first reported for *Methanobacterium bryantii* in the early



1970s (76). Since then several volatile methylated arsenicals (trimethyl arsine, dimethyl ethyl arsine, dimethyl arsine hydride, methyl arsine dihydride) have been detected in pure cultures as well as in anaerobic ecosystems. *Methanobacterium formicum* is efficient at producing methylated arsines and arsine; other anaerobes such as *Clostridium collagenovorans*, *Desulfovibrio gigas*, and *Desulfovibrio vulgaris* produce small amounts of trimethyl arsine (80). Enrichment cultures of anaerobic oligotrophs produce a high proportion of dimethyl arsenate (10). Sulfate-reducing consortia grown with acetate generate primarily TMAsO as well as methyl arsonate and dimethyl arsenate, with the relative proportion of the last two increasing with time. When these same consortia were grown with lactate, however, TMAsO was produced only by the fourth day along with a large number of unidentified products. Thus, product distribution is dependent not only on the microbe but also on the growth conditions.

Methylated arsenicals can be generated by different processes, as the source of the methyl group and the transfer reaction can vary. In fungi *S*-adenosylmethionine (SAM) serves as the donor, while anaerobic bacteria may use methyl cobalamin (29, 61, 136). Early studies with *M. bryantii* suggest that methyl transfer to arsenic is enzymatic (76). Although there is no consensus (16, 111) on the mechanism for anaerobic dimethyl arsine formation, in principle, there are three possibilities (Figure 3). The transfer of CH_3^+ can be mechanistically similar to that of SAM (i.e., reduction followed by oxidative addition; Figure 3, step 3). In this case a Co(I)^- species (B_{12s}) should be formed; however, the product detected after methyl transfer was B_{12r} with a paramagnetic Co(II) center (76). The detection of the paramagnetic species suggests that a free-radical mechanism is operative through homolytic bond cleavage of the metal (Co)-carbon bond (Figure 3, step 2). Thus, the bacterial methyl transfer reaction could be different from that proposed for eukaryotes. An alternative to direct methyl

group transfer is methyl group transfer involving an intermediate, possibly SAM. The role of methyl cobalamin in methylating SAM has not been unequivocally demonstrated in bacterial systems; however, both methionine synthase and methionine adenosyltransferase could be involved (35). In addition, a novel SAM-dependent pathway in rat liver has been discovered that involves a 42-kDa methyltransferase (designated cyt19) linked to thioredoxin-thioredoxin reductase (140). Its homolog, ArsM, has recently been identified in 125 Bacteria and 16 Archaea, and was characterized in *Rhodospseudomonas palustris* (104). ArsM not only conferred arsenic resistance, but also generates trimethyl arsine gas. Reduced glutathione was used as the two-electron donor (104).

Compared with the methylation reaction, even less is known about the mechanisms of demethylation. The first example of As-C bond cleavage was suggested in 1945 by Challenger (12), who reported that *Scopulariopsis brevicaulis* and *Penicillium notatum* produced trimethyl arsine from $\text{ClCH}_2\text{CH}_2\text{AsO}(\text{OH})_2$. It was suggested that ClCH_2CH_2 must have been removed after at least one methylation step because *P. notatum* does not methylate arsenate (12). The dealkylation of caddylic acid and sodium methane arsonate by soil microorganisms has been reported, and *Alcaligenes*, *Pseudomonas*, and *Mycobacterium* species have been shown to demethylate mono and dimethyl arsenic compounds (6). Maki et al. (73) have recently cultured *Pseudomonas* species that use DMA as a carbon source, releasing the arsenic in the process. The enzymes involved are completely unknown. Although the demethylation reaction has not

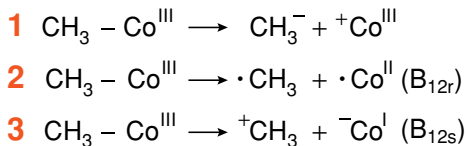


Figure 3

Proposed schemes for the methylation of arsenic involving cyanocobalamin (6).

been investigated at any length, if the reaction follows a “reverse Challenger” mechanism, it would involve reductive elimination and oxidation of the center. In this context, only methylated species with As(V) can be demethylated.

Organoarsenicals

The widespread use of organoarsenicals in agriculture has introduced another source of arsenic into the environment. Almost 2500 tons of herbicidal methylated arsenic compounds (e.g., MSMA, DSMA) are applied yearly to cotton crops, citrus groves, and golf courses. Several forms of organoarsenicals have been used as feed additives including 3-nitro-4-hydroxyphenylarsonic acid (roxarsone), *p*-arsanilic acid, 4-nitrophenylarsonic acid, and *p*-ureidophenylarsonic acid. All four are phenolic compounds with different side groups, and the arsenic is in its most oxidized state (As(V)). Roxarsone is readily extracted (70% to 90% recovery) in water and can be biodegraded (30, 116). Two pathways have been proposed for the degradation of roxarsone (81). In the first pathway, the arsenate is cleaved off and released into the environment, where it may be subsequently reduced [to As(III)] or methylated through yet another series of reactions (6). In the second pathway, the nitro group is first reduced and then removed via deamination. The 4-hydroxy phenylarsonic acid may also be subject to further transformation. Preliminary investigations on the decomposition of roxarsone in chicken litter have shown that inorganic arsenic is the predominant end product after prolonged composting (5, 30, 101). The mechanism by which the arsenic side group is released from the phenolic is currently unknown.

Arsenic Resistance

The arsenic resistance mechanism conferred by the *ars* operon has been extensively studied (as reviewed in References 82, 112, 131).

Originally described as plasmid borne, chromosomal loci have been found in more than 50 organisms including Archaea, Bacteria, yeasts, and protoctists (49). It is apparent that these three different but comparable systems (as exemplified by *Escherichia coli*, *Staphylococcus*, and yeast) have evolved through convergent evolution (131). Each system has two essential components: the arsenate reductase (ArsC, ACR2) and an arsenite-specific efflux pump (ArsB, ACR3). ArsC is a small-molecular-mass protein (13 to 15 kDa) related to a class of tyrosine phosphatases that mediates the reduction of As(V) to As(III) in the cytoplasm. Additional components include an ATPase (ArsA), regulatory elements (ArsR, ArsD), and a source of reducing equivalents (e.g., reduced thioredoxin or glutaredoxin). Other genes that have also been found in *ars* operons are *arsH* (67) and rhodanese (9); however, their functions, if any, in arsenic resistance have not been established.

The *ars* operon of plasmid R733 from *E. coli* is composed of *arsA*, *arsB*, *arsC*, *arsD*, and *arsR*, whereas the chromosomal locus has only *arsB*, *arsC*, and *arsR* (33, 75). Arsenate first binds to a triad of arginine residues (Arg-60, Arg-94, Arg-107) and then forms a covalent bond with a cysteine residue located near the N terminus (Cys12) (112). A disulfide bond is formed between the cysteine in ArsC and a cysteine in glutathione. Electrons pass via a cysteine on the glutaredoxin to reduce the disulfide and subsequently the As(V) (131). As(III) is then expelled from the cytoplasm through an ATP-dependent arsenite transporter formed by ArsAB (75). The system is regulated by ArsR and ArsD, *trans*-acting repressors that bind As(III) (13, 144). The yeast system is similar in that glutathione and glutaredoxin are involved. However, As(III) may be transported outside the cell by an ATP-independent exporter Acr2, or into a vacuole by Ycf1p (112).

The *ars* operon in plasmid pI258 of *Staphylococcus aureus* contains only *arsB*, *arsC*, and *arsD* (112, 131). In this case, arsenic is bound to an N-terminal cysteine; however, the two

additional cysteines required for the electron cascade are also part of the ArsC. Reduced thioredoxin provides the electrons to reduce As(V), and As(III) is expelled from the cell via an ATP-independent ArsB (112, 131).

The Ars resistance system is found in many prokaryotes and there can be many variations in gene number and order (11). Some species have multiple *ars* operons and tandem *arsC* genes (104). Recently, an aquaglycerol porin gene (*aqpS*), normally associated with arsenite import, was found in place of *arsB* in the *ars* operon of *Sinorhizobium meliloti* and functioned in arsenite export (145). Although arsenic resistance is not directly involved in arsenate respiration, *ars* operons have been found in arsenate-respiring bacteria (118–120). In some cases, the *arr* and *ars* operons lie in close proximity, suggestive of an “arsenic metabolism island,” but may be in *cis* or *trans* (120, 131). Many arsenite-oxidizing bacteria also have an *ars* operon, providing them the ability to both oxidize and reduce arsenic (55, 69). When genes associated with arsenite oxidation in *Agrobacterium tumefaciens* strain 5A were knocked out the mutants expressed an arsenate-reducing phenotype (56).

Respiratory Arsenate Reductase

To date, only the respiratory arsenate reductases (Arr) from *Chrysiogenes arsenatis* and *B. selenitireducens* have been purified and characterized (1, 60). The enzyme has also been identified in *Sbewanella* sp. strain ANA-3 through mutagenesis studies (119). The enzyme from *C. arsenatis* was found in the periplasm (60), whereas the enzyme purified from *B. selenitireducens* had to be solubilized from cell fractions with a detergent (CHAPS), indicating it is membrane associated (1). The optimum pH and salinity for activity of the Arr from *B. selenitireducens* were similar to that for growth of the organism (pH 9.8, NaCl 150 g l⁻¹), indicating an orientation of the catalytic subunit toward the periplasm rather than into the cytoplasm. The enzymes also

have a different affinity for arsenate, as the K_m of the Arr from *C. arsenatis* is 300 μ M but only 34 μ M for *B. selenitireducens*. The higher V_{max} of the Arr from *C. arsenatis* (7013 and 2.5 μ mol arsenate reduced min⁻¹ mg protein⁻¹) suggests that the enzyme compensates lower affinity with higher turnover (123). Substrate specificity is also different, as *C. arsenatis* Arr appears to be specific for arsenate (when reduced benzyl viologen is used as the artificial electron donor). *B. selenitireducens* Arr, however, can also reduce arsenite, selenate, and selenite (albeit at much lower activities) when reduced methyl viologen is provided as the artificial electron donor. In both cases, the enzymes were purified as a heterodimer, with a large catalytic subunit (ArrA) and a smaller electron transfer protein (ArrB). Metals analysis indicated the presence of iron and molybdenum. Direct comparison of the amino acid sequence of the catalytic subunit reveals only a 47% sequence identity and 77% similarity. Nevertheless, several regions show significant homology, especially the molybdenum-binding motif (**Figure 4**). Although no structural data are yet available, it is tempting to suggest, on the basis of sequence analysis with periplasmic nitrate reductase (20), that the molybdenum is ligated by a cysteinato sulfur (**Figure 4**).

Genetic and genomic analyses have provided a more comprehensive picture of Arr. The *arr* operon in *Sbewanella* sp. strain ANA-3 lies immediately downstream of the *ars* operon and contains only two genes, *arrA* and *arrB*. They encode proteins of predicted molecular masses of 95.2 kDa (ArrA) and 25.7 kDa (ArrB). ArrA contains motifs for binding an iron-sulfur cluster (C-X₂-C-X₃-C-X₂₇-C) and molybdenum-containing pyranopterin cofactor (**Figure 4**), as well as the Tat (twin-arginine translocation) signal sequence (S/T-R-R-X-F-L-K) at the N terminus (127). ArrB is predicted to contain three [4Fe-4S] and one [3Fe-4S] iron-sulfur clusters. Both *arrA* and *arrB* from *B. selenitireducens* have also been sequenced and show similar motifs (1, 105). Several research groups have since used

Crystal structures

TMAO reductase (<i>S.m.</i>)	GNYVKKIGDYSTGAGQTIL
DMSO reductase (<i>R.sp.</i>)	AGGFNSSGDYSTAAAQIIM
Periplasmic nitrate reductase (<i>D.d.</i>)	FGTNNVDGNPRLCMASAVG
Formate dehydrogenase O (<i>E.c.</i>)	LGMLAVDNQARVUHGPTVA
Nitrate reductase (<i>E.c.</i>)	GGTCLSFYDWYCDLPPASP
Arsenite oxidase (<i>A.f.</i>)	QTPMVRIHNRPAYNSECHA

Selenate reductase

Selenate reductase (<i>T.s.</i>)	GAIKPDVSSMTGDLYPGIQ
Chlorate reductase (<i>I.d.</i>)	GAI SPDATSM TGDLYTGIQ
Dimethylsulfide dehydrogenase (<i>R.su.</i>)	GGVQLDIFTDVGDLNTGAH
Ethylbenzene dehydrogenase (<i>A.sp.</i> EB1)	DGVSPDINVDIGDTYMGAF
Perchlorate reductase (<i>D.a.</i>)	GAHTHTFFDWYSDHPTGQT
Nitrate reductase (<i>E.c.</i>)	GGTCLSFYDWYCDLPPASP

Arsenite oxidase

<i>Aliccaligenes faecalis</i>	QTPMVRIHNRPAYNSECHA
<i>Cenibacterium arsenoxidans</i>	QTPTVRIHNRPAYNSECHA
<i>Rhodoferrax ferrireducens</i>	QTPMVRIHNRPAYNSECHA
<i>Nitrobacter hamburgensis</i>	KVKNIRIHNRPAYNSEVHA
NT26	KVKNIRIHNRPAYNSEVHG
<i>Chlorobium phaeobacteroides</i>	KTQYLSIHNRPAYNSETWG
<i>Chlorobium limicola</i>	KTNYLSIHNRPAYNSETWG
<i>Chloroflexus aurantiacus</i>	QTINCSIHNRPAYNSEVHA
<i>Thermus thermophilus</i>	SVKHIAIHNRPAYNSEVWG

Arsenate reductase

<i>Chysioyenes arsenatis</i>	GSPNNISHSAICAEVEKMG
<i>Desulfobacterium hafniense</i>	GSPNNISHSSI CAEAEKFG
<i>Clostridium sp.</i> OhLAs	GSPNNISHSSI CAEAEKFG
<i>Alkaliphilus metalliredigens</i>	GSPNNISHSSI CAEAEKFG
<i>Bacillus selenitireducens</i>	GSPNNISHSSI CAESEKFG
<i>Bacillus areniciselenatis</i>	GSPNNVSHSSI CAETEKHG
<i>Desulfosporosinus sp.</i> Y5	GSPNNISHSAICAEAEKFG
<i>Shewanella sp.</i> ANA-3	GSPNNISHSSVCAEAHKMG
<i>Wolinella succinogenes</i>	GSPNNISHSSI CAEAEKFG
<i>Sulfurospirillum barnesii</i>	GSPNNISHSASICAKTEKFG
MLMS1	GSPNNISHSSI CAEAEKMG

Figure 4

Amino acid sequence alignment of the molybdenum-binding domain. The amino acid that coordinates to the molybdenum (highlighted in orange) may be serine (TMAO and DMSO reductase), cysteine (periplasmic nitrate reductase), selenocysteine (formate dehydrogenase), or aspartic acid (respiratory nitrate reductase). The exception is arsenite oxidase, where alanine should coordinate to the metal, but does not (highlighted in blue). We predict, on the basis of sequence analysis, that the amino acid that coordinates to the molybdenum in selenate and chlorate reductase is aspartic acid, and in arsenate reductase is cysteine (highlighted in orange). Gray, green, and yellow shaded areas indicate conserved amino acids. Abbreviations: *A.sp.* EB1, *Azoarcus sp.* EB1; *A.f.*, *Aliccaligenes faecalis*; *D.d.*, *Desulfobacterium desulfuricans*; *D.a.*, *Dechloromonas agitata*; *E.c.*, *Escherichia coli*; *I.d.*, *Ideonella dechloratans*; *R.sp.*, *Rhodobacter sphaeroides*; *R.su.*, *Rhodovulum sulfidophilum*; *S.m.*, *Shewanella massilia*; *T.s.*, *Thaueria selenatis*.

degenerate primers to amplify (by PCR) and sequence a major portion of *arrA* from other bacterial species including *B. arseniciselenatis*, *C. arsenatis*, and *Sulfurospirillum barnesii* (74), and *Desulfosporosinus* sp. strain Y5 (102). The *arr* operon has been identified by homology in the genomes of the arsenate-respiring bacteria *Desulfitobacterium hafniense* (*arrSKRCAB*), *Wolinella succinogenes* (*arrCAB*), *Alkaliphilus metalliredigens* (*arrCAB*), and more recently from *Clostridium* species strain OhILAs (*arrCAB*) and Mono Lake isolate MLMS-1 (*arrSKRCAB*). Phylogenetic analysis of ArrA reveals that they are most similar to each other but also share similarities to the catalytic subunit of the polysulfide reductase (PsrA) of *W. succinogenes* and *Shewanella oneidensis*, and the catalytic subunit of thiosulfate reductase (PhsA) of *Salmonella typhimurium* (Figure 5). The group forms a cohesive clade in the DMSO reductase family of molybdenum enzymes. More importantly, the genome data have revealed heterogeneity in the number of genes encoding structural proteins and regulatory elements. *D. hafniense*, *W. succinogenes*, *A. metalliredigens*, *Clostridium* sp. OhILAs, and MLMS-1 possess a membrane-integral third subunit (ArrC). Although *D. hafniense* and MLMS-1 have genes indicative of a two-component regulatory system upstream of *arrCAB*, *W. succinogenes* and *A. metalliredigens* apparently do not.

Arsenite Oxidase

Arsenite oxidase (Aox) is also a member of the DMSO reductase family of molybdenum enzymes, but it is not directly related to Arr (Figure 5). Aox from *Alcaligenes faecalis* has been purified (4) and structurally characterized (26). A similar enzyme has also been purified from the heterotrophic arsenite oxidizer *Hydrogenophaga* sp. strain NT-14 (143) and the chemolithoautotrophic *Rhizobium* sp. strain NT-26 (125). A heterodimer, Aox is comprised of the catalytic subunit (AoxB, ~90 kDa) that contains a [3Fe-4S] cluster and a molybdenum bound to the pyranopterin

cofactor. Crystal structure indicates that unlike other members of the DMSO reductase family, no amino acid coordinates to the molybdenum, but that an alanine residue lies at that position (26). The molybdenum is a dioxo form coordinated by four sulfur ligands in the oxidized form of the enzyme (14) that is reduced (and reoxidized) by a coordinated two-electron transfer (45). The associated subunit (AoxA, ~14 kDa) has a single Rieske-type [2Fe-2S] cluster, a feature that is unique among molybdenum enzymes (26). Another unique feature is that the small subunit has the TAT leader sequence. The genes encoding the two subunits have been cloned and sequenced by different groups and unfortunately were given different names. The gene designation *aox* is synonymous with *aro* and *aso*. To further complicate the matter, the gene encoding the catalytic subunit was designated *aoxB* in *Cenibacterium arsenoxidans* strain ULPA1 (83) and *Agrobacterium tumefaciens* (55), *asoA* in *Acaligenes faecalis* (131), and *aroA* in *Rhizobium* sp. strain NT-26 (125). Regardless of nomenclature, the proteins are homologous.

As was observed in the *arr* operons, there is also heterogeneity in the *aro* operons. Whereas the order *aoxAB* is conserved, other elements are generally not (131). An identical gene arrangement (*aoxRSABC-moeA*), however, has been identified in the Aox gene clusters of NT-26 (J. M. Santini, unpublished) and *Agrobacterium tumefaciens* (55). BLAST searches of available genome-sequencing data have revealed potential arsenite oxidases (64) including two Crenarchaeota *Aeropyrum pernix* and *Sulfolobus tokodaii*, thermophilic Bacteria (*Thermus thermophilus* strain HB8), anoxyphototrophic bacteria (*Chloroflexus aurantiacus*, *Chlorobium limicola*, *Chlorobium phaeobacteroides*), as well as *Nitrobacter hamburgensis*, and *Rhodospirillum rubrum* (Figure 5). While it is often premature to assign a physiological function to a protein identified solely on sequence homology, the high degree of sequence similarity (particularly residues within the region

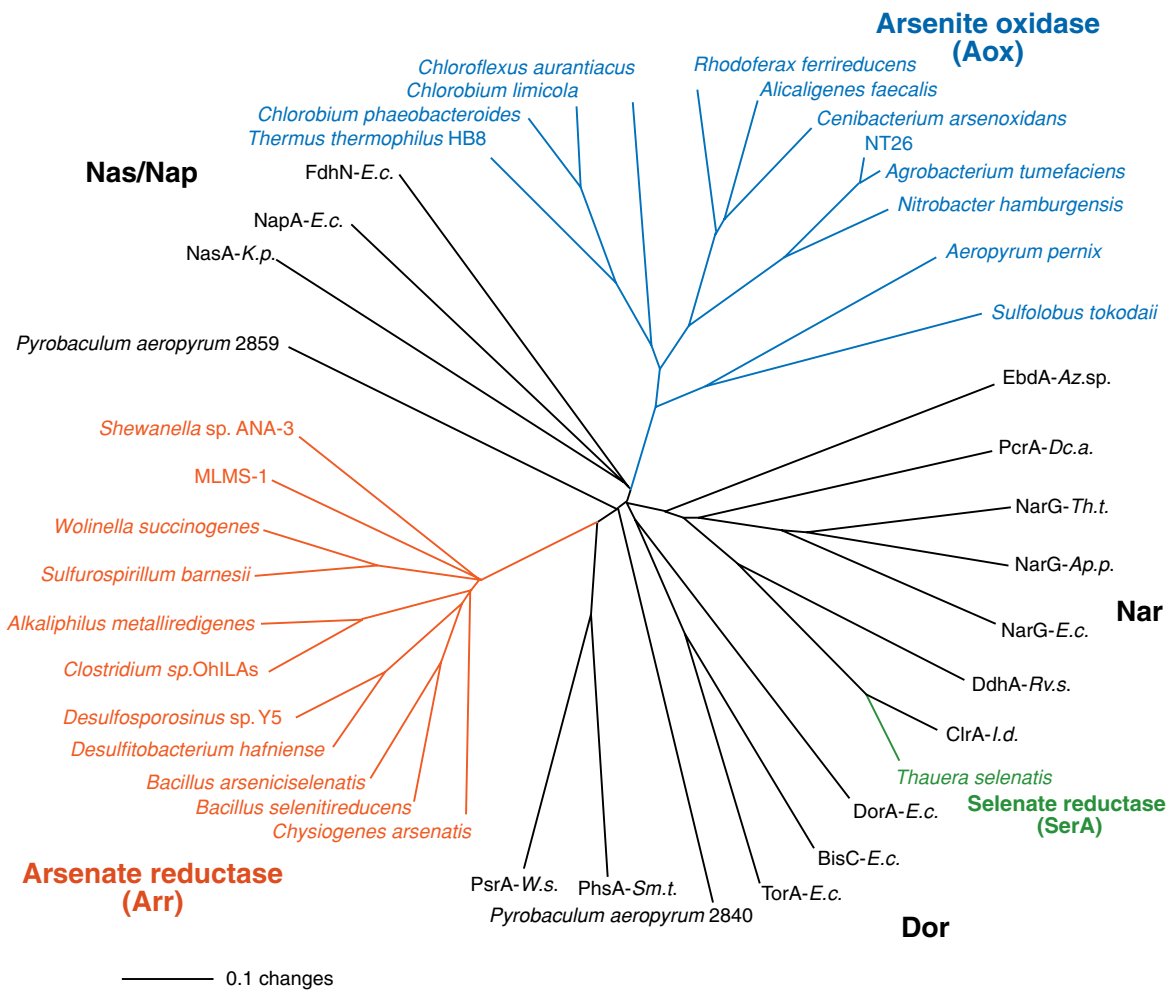


Figure 5

Phylogenetic tree (unrooted neighbor joining) of the DMSO reductase family of molybdenum enzymes. Arsenate reductase (Arr, orange) and arsenite oxidase (Aox, blue) define separate and distinct branches. The other major clades in the family are DMSO reductase (Dor), assimilatory and periplasmic nitrate reductases (Nas/Nap), and respiratory nitrate reductase (Nar) in which the respiratory selenate reductase (SerA, green) belongs. Abbreviations are the same as in **Figure 4**; in addition, *Ap.p.*, *Aeropyrum pernix*; *K.p.*, *Klebsiella pneumoniae*; *Sm.t.*, *Salmonella typhimurium*; *Th.t.*, *Thermus thermophilus*; *W.s.*, *Wolinella succinogenes*. *Pyrobaculum aeropyrum* 2859 and 2840 are putative Arr homologs.

comprising the active site) is significant (**Figure 4**). Interestingly, the branching of the AoxB appears to follow 16S rRNA gene-based phylogenetic lineages, supporting the idea of an ancient origin for this enzyme (64).

SELENIUM IN CELLULAR METABOLISM

Selenium is an analog of sulfur and may substitute for sulfur in thiols. Thus it can be toxic at elevated concentrations. Nevertheless, it is an essential element for microorganisms

(39). The requirement for selenite by *E. coli* in the production of formate dehydrogenase was recognized in the mid-1950s (103); however, it took several more decades before the precise nature of the requirement was discovered. The common biological forms are selenocysteine (the twenty-first amino acid) and selenomethionine. Selenocysteine is encoded by its own tRNA and provides the selenium in glycine reductase, formate dehydrogenase, and NiFeSe hydrogenase (31, 39). Selenium is assimilated in yeast and plants via the sulfur assimilation pathway with selenate activated by ATP sulfurylase and subsequently converted to selenomethionine (19). Although a similar mechanism has been proposed for prokaryotes, some studies have suggested specific mechanisms for the uptake of selenate and selenite (135). The reaction of selenite with glutathione produces selenodiglutathione. Selenodiglutathione and its subsequent reduction to glutathioselenol are key intermediates in the transformation of selenium (142).

The use of selenium oxyanions as alternative terminal electron acceptors is energetically favorable. The free energies when coupled with H₂ oxidation are significant, with values of $-15.53 \text{ kcal mol}^{-1} \text{ e}^{-}$ for SeO_4^{2-} and $-8.93 \text{ kcal mol}^{-1} \text{ e}^{-}$ for HSeO_3^{-} (85). Although Se(IV) reduction to Se(0) also occurs in nature, only the haloalkaliphilic *B. selenitireducens* (137) and three strains of an *Aquificales* species (HGMK-1, 2, and 3) (139) that can respire selenite have been described. No respiratory selenite reductase has been reported to date. Selenium reduction to selenide (HSe^{-1}) has only recently been described (42). Far less is known about selenium oxidation. Studies on microbial selenium methylation and demethylation are also few in number (25, 27, 29, 77, 100, 106, 107). Thus, many aspects of the biogeochemical cycle of selenium remain to be explored.

Selenocysteine

Selenium is incorporated into proteins primarily in the form of selenocysteine (Sec, U).

Sec is the twenty-first amino acid and uses the UGA codon (132). Four gene products are directly involved in the biogenesis of Sec proteins: Sela, selenocysteine synthase; SelB, a special translation factor that binds guanine nucleotides and specifically recognizes selenocystyl-tRNA^{Sec}; SelC, a Sec-specific tRNA (tRNA^{Sec}); and SelD, the selenophosphate synthase (8, 24). Selenocysteine is not ligated directly to tRNA^{Sec} but rather derived from an L-serine. Sela converts seryl-tRNA^{Sec} to aminoacrylyl-tRNA^{Sec}. The aminoacrylyl-tRNA^{Sec} is then converted to selenocystyl-tRNA^{Sec} with activated monoselenophosphate generated by SelD. SelB recognizes the alternative coding of UGA for selenocysteine aided by a stem loop structure in the mRNA (115). The selenocysteine insertion sequence, or SECIS, is found at the 3' untranslated region of the mRNA in Archaea and eukaryotes (23, 28, 114). In Bacteria, the SECIS is found immediately downstream of the UGA codon within the open reading frame.

Selenoproteins found in Bacteria include the three forms of formate dehydrogenase (FdhN, FdhO, FdhH) found in *E. coli*, glycine reductase A and glycine reductase B found in *Clostridium* species, and NiFeSe hydrogenase found in *Desulfovibrio vulgaris*. In addition to formate dehydrogenase, methanogens may have selenium-containing forms of heterodisulfide reductase, formylmethanofuran dehydrogenase, the F₄₂₀-nonreducing hydrogenase (two subunits), and F₄₂₀-reducing hydrogenase (113). Interestingly, cysteine homologs of these enzymes also exist. *Methanococcus maripaludis* has a single copy of the genes encoding the selenocysteine homologs of the F₄₂₀-reducing hydrogenase and the two subunits of the F₄₂₀ nonreducing hydrogenase. Thus methanogenesis in this organism is selenium dependent. *Methanococcus voltae* has additional genes that encode for cysteine-containing homologs of the two hydrogenases (36) and is thus facultative with respect to selenium dependence (113). The expression of the different homologs is

regulated by selenium by an as yet undetermined mechanism (84).

The identification of selenoproteins in silico is complicated by several factors (23, 62). First, many of the current bioinformatics tools recognize UGA only as a stop codon. At the time this review was written, BLAST searches were still incapable of handling selenoenzymes, as the program automatically replaced the U with an X. Second, there are no specific locations for selenocysteine. When it occurs close to the N terminus, the resulting truncated protein is usually unrecognized, as the size falls below the threshold for an open reading frame. Premature termination of a protein sequence can result when selenocysteine lies close to the actual C terminus, as is the case with some thioredoxin reductases (23). There are also examples of proteins that contain multiple selenocysteines (e.g., mammalian SelP) (23). The greatest difficulty, however, is that many of these proteins have cysteine-containing homologs, resulting in incorrect annotation. Although *E. coli* has three selenium-containing formate dehydrogenases, these enzymes belong to a much larger group of enzymes, the DMSO reductase of the molybdenum-containing enzyme family (**Figure 5**). Sequence analysis of the other members in this family indicates that none are selenoproteins. Regardless, the common annotation in vogue is “anaerobic dehydrogenases typically selenocysteine-containing.” The identification of bona fide selenoproteins needs to be verified through proteomics.

On a positive note, more selenoproteins are being recognized. Sec-Fdh homologs have been identified in the genomes of *Desulfovibrio vulgaris*, *Geobacter metallireducens*, and *Campylobacter* species (*C. jejuni*, *C. upsaliensis*). Glycine reductase homologs have been identified in the genomes of *Clostridium*, *Eubacterium*, *Desulfuromonas*, *Carboxydotherrmus hydrogenoformans*, *Photobacterium*, *Symbiobacterium*, and even *Treponema*. Selenocysteine-containing homologs of thioredoxin and

glutaredoxin have been identified in the genome of *Geobacter sulfurreducens*. A BLAST search of SelA and SelB done for this review, however, yielded hits in 50 genomes including those of *Aquifex aeolicus*, *Sinorhizobium meliloti*, *Burkholderia pseudomallei*, *Salmonella enterica*, *Shewanella oneidensis*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Shigella dysenteriae*, *Yersinia pseudotuberculosis*, and *Helicobacter hepaticus*, suggesting that selenoproteins are more ubiquitous.

Methylation

Dimethyl selenide and dimethyl diselenide are the most common forms of methylated selenium. In both cases, the selenium is in its fully reduced oxidation state (Se-II). This is unlike arsenic, in which the methylated species may contain arsenic in the V, III, or -III state. As with arsenic, methylation via a methyltransferase is a common mode of removal in prokaryotes (39). *Rhodocyclus tenuis* and *Rhodospirillum rubrum* produce both dimethyl selenide and dimethyl diselenide from selenate while growing phototrophically (77). *R. tenuis* also produces dimethyl selenide from selenite (77). Selenocysteine can be reduced to hydrogen selenide by reduced glutathione via selenocysteine-glutathione selenenyl sulfide (128). *E. coli* cells expressing the trimethyl purine methylase gene (*tmp*) from *Pseudomonas syringae* produced dimethyl selenide and dimethyl diselenide from selenate, selenite, and selenocysteine (108). Recently, two different methyltransferases have been identified from species of *Pseudomonas* (106, 107). The first is a bacterial thio-purine methyltransferase. The enzyme converts selenite and selenocysteine to dimethyl selenide and dimethyl diselenide (106). The second, a homolog of calichaemicin methyltransferase, produces dimethyl selenide and dimethyl diselenide from selenite and selenocysteine (107). The enzyme, MmtA, is defining a new group of methyltransferases and has homologs in many species of Bacteria (107).

Respiratory Selenate Reductase

Currently, only the respiratory selenate reductase (Ser) from *Thauera selenatis* has been studied in detail. The enzyme has been purified and characterized (72, 129) and the genes have been cloned and sequenced (59). Ser is a heterotrimer consisting of three heterologous subunits, SerA (96 kDa), SerB (40 kDa), and SerC (23 kDa), with a native molecular weight of 180 kDa. It has a high affinity for selenate with a K_m of 16 μ M and does not use selenite, nitrate, nitrite, sulfate (129), arsenate, or arsenite (S. Bydder & J. M. Santini, unpublished data) as substrates. Ser contains molybdenum, iron, acid-labile sulfur, and heme *b* (129). The visible spectrum of the reduced heme *b* has absorbance maxima at 425 (soret), 528 (β), and 558 (α) nm. X-ray absorption spectroscopy suggests that the molybdenum is coordinated by four sulfur ligands, with the oxidized form having a dioxo and the reduced form having a des-oxo form of the molybdenum (72). Maher et al. (72) also found evidence for selenium in the enzyme; however, the inferred amino acid sequence has neither the UGA codon nor the SECIS.

The *ser* operon contains four open reading frames, *serABDC*. The catalytic subunit, SerA, has a TAT leader sequence, indicating its transport to the periplasm (127) and the modified [4Fe-4S] cluster binding motif at the N terminus (7). It falls in with other enzymes of the DMSO reductase family (79) (**Figure 5**) and is most closely related, on the basis of sequence alignment, to the chlorate reductase (ClrA, 84% identity and 96% similarity) from *Ideonella dechloratans*, dimethyl sulfide dehydrogenase (DdhA, 43% and 62%) from *Rhodovulum sulfidophilum*, and ethylbenzene dehydrogenase (EbdA, 31% and 47%) from *Azoarcus* sp. EB 1. These enzymes all group with the NarG nitrate reductase, and all four proteins (SerA, ClrA, DdhA, EbdA) as well as the perchlorate reductase (PcrA) from *Dechloromonas agitata* have an aspartate residue in the same position (**Figure 4**). This residue (Asp222) has been shown by crystal

structure to be coordinated to the molybdenum in NarG. A direct comparison of the nucleic acid sequence of the *ser* and *chl* operons revealed a high degree of identity (81.7%). The gene order is the same and the proteins encoded also have a high degree of identity: SerA/ChlA (83%), SerB/ChlB (91%), SerC/ChlC (76%), SerD/ChlD (79%). Regardless, chlorate reductase is a poor selenate reductase (141). This observation suggests that the function of a protein may not always be ascertained simply by gene or amino acid sequence analysis and must be confirmed by physiological and molecular studies.

SerB shares similarities to the Fe-S proteins of Clr, Ddh, Ebd, and Nar. SerB contains four cysteine motifs that may function in binding 4[Fe-S] clusters. These motifs are also conserved in ClrB, DdhB, EbdB, and NarH. NarH contains a tryptophan in place of the tyrosine and this cluster coordinates a [3Fe-4S] cluster and the other three coordinate 3[4Fe-4S] clusters (7). SerC contains a leader sequence, suggesting that it is exported to the periplasm in an unfolded state. SerC is probably a *b*-type cytochrome, as it has two conserved amino acids, histidine and methionine (78). SerD is not part of the purified Ser and is similar to ClrD, DdhD, and EbdD; its function is not known. Similar to NarJ, it may act as a molecular chaperone and in protein maturation (123).

CONCLUSIONS AND FUTURE DIRECTIONS

This brief review has highlighted the recent advances in our understanding of selenium and arsenic in microbial metabolism. While these two chemical elements are often considered together, there are clear differences in their roles in cellular metabolism. Selenium is an essential trace element and is incorporated into an amino acid (selenocysteine), but this is not the case for arsenic. The respiratory enzymes belong to the same family of enzymes,

but Ser is more similar to a nitrate reductase, and Arr, oddly enough, is more similar to a thiosulfate reductase. The relative ubiquity of pathways and processes involving these two elements in Archaea and Bacteria indicates they have been important selective factors in microbial evolution. The increasing availability of genome data will help researchers explore metabolic diversity in these organisms, possibly leading to the discovery of new pathways and regulatory elements. Some of the examples we have provided here, however,

assuage caution in interpretation, as function cannot always be inferred from sequence homology (e.g., Ser/Chl), underscoring the need to verify annotation by physiological, biochemical, and molecular studies. Better tools for in silico identification of selenoproteins are sorely needed. Additional key areas in need of further investigation are the processes and enzymes involved in methylation and demethylation of both arsenic and selenium, and the identification of selenite and selenide reductases.

SUMMARY POINTS

1. Arsenic and selenium have biogeochemical cycles and can drive the ecology in certain environments.
2. Arr, Aox, and Ser are members of the DMSO reductase family but cluster with different enzymes.
3. Whereas the core enzymes are conserved, the operon organization, regulation, and additional subunit composition may vary.
4. The basic mechanisms for selenium incorporation into selenocysteine and the recognition of UGA as a Sec codon have been elucidated.

FUTURE ISSUES

1. Better tools for the in silico identification of selenoproteins are needed.
2. Further investigation is needed on selenium respiration including the identification of a selenite reductase.
3. Further investigation is needed on the transformation of organoarsenicals and the processes of methylation and demethylation.

LITERATURE CITED

1. Afkar E, Lisak J, Saltikov C, Basu P, Oremland RS, Stolz JF. 2003. The respiratory arsenate reductase from *Bacillus selenitireducens* strain MLS10. *FEMS Microbiol. Lett.* 226:107–12
2. Ahmann D, Krumholz LR, Hemond H, Lovley DR, Morel FMM. 1997. Microbial mobilization of arsenic from sediments of the Aberjona Watershed. *Environ. Sci. Technol.* 31:2923–30
3. Ahmann D, Roberts AL, Krumholz LR, Morel FMM. 1994. Microbe grows by reducing arsenic. *Nature* 371:750
4. Anderson GL, Williams J, Hille R. 1992. The purification and characterization of arsenite

3. First report of an organism capable of respiring arsenic.

- oxidase from *Alcaligenes faecalis*, a molybdenum-containing hydroxylase. *J. Biol. Chem.* 267:23674–82
5. Arai Y, Lanzirotti A, Sutton S, Davis JA, Sparks DL. 2003. Arsenic speciation and reactivity in poultry litter. *Environ. Sci. Technol.* 37:4083–90
 6. Bentley R, Chasteen TG. 2002. Microbial methylation of metalloids: arsenic, antimony, and bismuth. *Microbiol. Mol. Biol. Rev.* 66:250–71
 7. Bertero MG, Rothery RA, Palak M, Hou C, Lim C, et al. 2003. Insights into the respiratory electron transfer pathway from the structure of nitrate reductase A. *Nat. Struct. Biol.* 10:681–87
 8. Böck A. 2000. Biosynthesis of selenoproteins: an overview. *Biofactors* 11:77–78
 9. Bordo D, Bork P. 2002. The rhodanese/Cdc25 phosphatase superfamily: sequence-structure-function relations. *EMBO Rep.* 3:741–46
 10. Bright DA, Brock S, Cullen WR, Hewitt GM, Jafaar J, Reimer KJ. 1994. Methylation of arsenic by anaerobic microbial consortia isolated from a lake sediment. *Appl. Organomet. Chem.* 8:415–22
 11. Butcher BG, Deane SM, Rawlings DE. 2000. The chromosomal arsenic resistance genes of *Thiobacillus ferrooxidans* have an unusual arrangement and confer increased arsenic and antimony resistance to *Escherichia coli*. *Appl. Environ. Microbiol.* 66:1826–33
 - 12. Challenger F. 1945. Biological methylation. *Chem. Rev.* 36:315–61**
 13. Chen Y, Rosen BP. 1997. Metalloregulatory properties of the ArsD repressor. *J. Biol. Chem.* 272:14257–62
 14. Conrads T, Hemann C, George GN, Pickering IJ, Prince RC, Hille R. 2002. The active site of the arsenite oxidase of *Alcaligenes faecalis*. *J. Am. Chem. Soc.* 124:11276–77
 15. Croal LR, Galnick JA, Malasarn D, Newman DK. 2004. The genetics of geochemistry. *Annu. Rev. Genet.* 38:175–202
 16. Cullen WR, Reimer KJ. 1989. Arsenic speciation in the environment. *Chem. Rev.* 89:713–64
 17. Cutter G. 1982. Selenium in reducing waters. *Science* 217:829–31
 18. Cutter GA, Bruland KW. 1984. The marine biogeochemistry of selenium: a re-evaluation. *Limnol. Oceanogr.* 29:1179–92
 19. de Souza MP, Lytle CM, Mulholland MM, Otte ML, Terry N. 2000. Selenium assimilation and volatilization from dimethylselenoniopropionate by Indian mustard. *Plant Physiol.* 122:1281–88
 20. Dias JM, Than ME, Humm A, Huber R, Bourenkov GP, et al. 1999. Crystal structure of the first dissimilatory nitrate reductase at 1.9 Å solved by MAD methods. *Structure* 7:65–79
 21. Dombrowski PM, Long W, Parley KJ, Mahony JD, Capitani JF, Di Toro DM. 2005. Thermodynamic analysis of arsenic methylation. *Environ. Sci. Technol.* 39:2169–76
 22. Dowdle PR, Oremland RS. 1998. Microbial oxidation of elemental selenium in soil slurries and bacterial cultures. *Environ. Sci. Technol.* 32:3749–55
 23. Driscoll DM, Chavatt L. 2004. Finding needles in a haystack: in silico identification of eukaryotic selenoprotein genes. *EMBO Rep.* 5:140–41
 24. Driscoll DM, Copeland PR. 2003. Mechanism and regulation of selenoprotein synthesis. *Annu. Rev. Nutr.* 23:17–40
 25. Dungan RS, Frankenberger WTJ. 1999. Microbial transformations of selenium and the bioremediation of seleniferous environments. *Bioremediation J.* 3:171–88
 26. Ellis PJ, Conrads T, Hille R, Kuhn P. 2001. Crystal structure of the 100 kDa arsenite oxidase from *Alcaligenes faecalis* in two crystal forms at 1.64 and 2.06 Å. *Structure* 9:125–32

12. Classic paper on biological methylation of inorganic compounds including arsenic.

**39. Classic review
on selenium in
biological systems.**

27. Favre-Bonte S, Ranjard L, Colinon C, Prigent-Combaret C, Nazaret S, Cournoyer B. 2005. Freshwater selenium-methylating bacterial thiopurine methyltransferases: diversity and molecular phylogeny. *Environ. Microbiol.* 7:153–64
28. Fourmy D, Guittet E, Yoshizawa S. 2002. Structure of prokaryotic SECIS mRNA hairpin and its interaction with elongation factor SelB. *J. Mol. Biol.* 324:137–50
29. Gadd GM. 1993. Microbial formation and transformation of organometallic and organometalloid compounds. *FEMS Microbiol. Rev.* 11:297–316
30. Garbarino JR, Bednar AJ, Rutherford DW, Beyer RS, Wershaw RL. 2003. Environmental fate of roxarsone in poultry litter. I. Degrading of roxarsone during composting. *Environ. Sci. Technol.* 37:1509–14
31. Garcin E, Vernede X, Hatchikian EC, Volbeda A, Frey M, Fontecilla-Camps JC. 1999. The crystal structure of a reduced [NiFeSe] hydrogenase provides an image of the activated catalytic center. *Struct. Fold. Des.* 7:557–66
32. Gihring TM, Banfield JF. 2001. Arsenite oxidation and arsenate respiration by a new *Thermus* isolate. *FEMS Microbiol. Lett.* 204:335–40
33. Gladysheva TB, Oden KL, Rosen BP. 1994. Properties of the arsenate reductase of plasmid R773. *Biochemistry* 33:7288–93
34. Green HH. 1918. Description of a bacterium which oxidizes arsenite to arsenate, and one of which reduces arsenate to arsenite, isolated from a cattle-dipping tank. *S. Afr. J. Sci.* 14:465–67
35. Gruber K, Kratky C. 2001. Cobalamin-dependent methionine synthase. *Handb. Metalloproteins* 2:1010–22
36. Halboth S, Klein A. 1992. *Methanococcus voltae* harbors four gene clusters potentially encoding two [NiFe] and two [NiFeSe] hydrogenases, each of the cofactor F420-reducing or F420-nonreducing types. *Mol. Gen. Genet.* 233:217–24
37. Harvey CF, Swartz CH, Badruzzaman ABM, Keon-Blute N, You W, et al. 2002. Arsenic mobility and groundwater extraction in Bangladesh. *Science* 298:1602–6
38. Hattori H, Kimura M, Nakaguchi Y, Hiraki K. 1999. Biogeochemical study on selenium in the Indian Ocean. *Kinki Daigaku Rikogakubu Kenkyu Hokoku* 35:73–78
39. Heider J, Böck A. 1993. **Selenium metabolism in microorganisms.** *Adv. Physiol.* 35:74–107
40. Herbel MJ, Johnson TM, Oremland RS, Bullen TD. 2000. Fractionation of selenium isotopes during bacterial respiratory reduction of selenium oxyanions. *Geochim. Cosmochim. Acta* 64:3701–9
41. Herbel MJ, Johnson TM, Tanji KK, Gao S, Bullen TD. 2002. Selenium stable isotope ratios in California agricultural drainage water management systems. *J. Environ. Qual.* 31:1146–56
42. Herbel MJ, Switzer Blum J, Oremland RS, Borglin SE. 2003. Reduction of elemental selenium to selenide: experiments with anoxic sediments and bacteria that respire Se-oxyanions. *Geomicrobiol. J.* 20:587–602
43. Hoefl SE, Kulp TR, Stolz JF, Hollibaugh T, Oremland RS. 2004. Dissimilatory arsenate reduction with sulfide as electron donor: experiments with Mono Lake water and isolation of strain MLMS-1, a chemoautotrophic arsenate respirer. *Appl. Environ. Microbiol.* 70:2741–47
44. Hoefl SE, Lucas F, Hollibaugh JT, Oremland RS. 2002. Characterization of microbial arsenate reduction in the anoxic bottom waters of Mono Lake, California. *Geomicrobiol. J.* 19:23–40

45. Hoke KR, Cobb N, Armstrong FA, Hille R. 2004. Electrochemical studies of arsenite oxidase: an unusual example of a highly cooperative two-electron molybdenum center. *Biochemistry* 43:1667–74
46. Hollibaugh JT, Carini S, Gürleyük H, Jellison R, Joye SB, et al. 2005. Arsenic speciation in Mono Lake, California: response to seasonal stratification and anoxia. *Geochim. Cosmochim. Acta* 69:1925–37
47. Huber R, Sacher M, Vollmann A, Huber H, Rose D. 2000. Respiration of arsenate and selenate by hyperthermophilic Archaea. *Syst. Appl. Microbiol.* 23:305–14
48. Islam FS, Gault AG, Boothman C, Polya DA, Chatterjee D, Lloyd JR. 2004. Direct evidence of arsenic release from Bengali sediments due to metal-reducing bacteria. *Nature* 430:68–71
49. Jackson CR, Dugas SL. 2003. Phylogenetic analysis of bacterial and archaeal *arsC* gene sequences suggests an ancient, common origin for arsenate reductase. *BMC Evol. Biol.* 3:18–27
50. Jackson CR, Langner HW, Donahoe-Christiansen J, Inskeep WP, McDermott TR. 2001. Molecular analysis of microbial community structure in an arsenite-oxidizing acidic thermal spring. *Environ. Microbiol.* 3:532–42
51. Jensen A, Finster K. 2005. Isolation and characterization of *Sulfurospirillum carboxydovovans* sp. nov., a new microaerophilic carbon monoxide oxidizing epsilon Proteobacterium. *Antonie van Leeuwenhoek* 87:339–53
52. Johnson TM, Bullen TD, Zawislanski PT. 2000. Selenium stable isotope ratios as indicators of sources and cycling of selenium: results from the northern reach of San Francisco Bay. *Environ. Sci. Technol.* 34:2075–79
53. Johnson TM, Herbel MJ, Bullen TD, Zawislanski PT. 1999. Selenium isotope ratios as indicators of selenium sources and biogeochemical cycling. *Geochim. Cosmochim. Acta* 63:2775–83
54. Jones CA, Langner HW, Anderson K, McDermott TR, Inskeep WP. 2000. Rates of microbially mediated arsenate reduction and solubilization. *Soil Sci. Soc. Am. J.* 64:600–8
55. Kashyap DR, Botero LM, Franck WL, Hassett DJ, McDermott TR. 2006. Complex regulation of arsenite oxidation in *Agrobacterium tumefaciens*. *J. Bacteriol.* 188:1081–88
56. Kashyap DR, Botero LM, Lehr C, Hassett DJ, McDermott TR. 2006. A Na⁺:H⁺ antiporter and molybdate transporter are essential for arsenite oxidation in *Agrobacterium tumefaciens*. *J. Bacteriol.* 188:1577–84
57. Kazufumi T, Wong GTF. 1985. Dissolved inorganic and organic selenium in the Orca Basin. *Geochim. Cosmochim. Acta* 49:539–46
58. Kiene R, Oremland RS, Catena A, Miller L, Capone DG. 1986. Metabolism of reduced methylated sulfur compounds in anaerobic sediments and by a pure culture of an estuarine methanogen. *Appl. Environ. Microbiol.* 52:1037–45
59. Krafft T, Bowen A, Theis F, Macy JM. 2000. Cloning and sequencing of the genes encoding the periplasmic-cytochrome *b*-containing selenate reductase of *Thauera selenatis*. *DNA Sequation* 10:365–77
- 60. Krafft T, Macy JM. 1998. Purification and characterization of the respiratory arsenate reductase of *Chrysiogenes arsenatis*. *Eur. J. Biochem.* 255:647–53**
61. Kräutler B. 1990. Chemistry of methylcorrinoids related to their roles in bacterial C1 metabolism. *FEMS Microbiol. Rev.* 87:349–54
62. Kryukov GV, Gladyshev VN. 2004. The prokaryotic selenoproteome. *EMBO Rep.* 5:538–43

60. Purification and characterization of the first respiratory arsenate reductase.

63. Laverman AM, Switzer Blum J, Schaefer JK, Phillips EJP, Lovley DR, Oremland RS. 1995. Growth of strain SES-3 with arsenate and other diverse electron acceptors. *Appl. Environ. Microbiol.* 61:3556–61
64. Lebrun E, Brugna M, Baymann F, Muller D, Lievreumont D, et al. 2003. Arsenite oxidase, an ancient bioenergetic enzyme. *Mol. Biol. Evol.* 20:686–93
65. Lipman JG, Waxman SA. 1923. The oxidation of selenium by a new group of autotrophic microorganisms. *Science* 57:60–61
66. Liu A, Garcia-Dominguez E, Rhine ED, Young LY. 2004. A novel arsenate respiring isolate that can utilize aromatic substances. *FEMS Microbiol. Ecol.* 48:323–32
67. Lopez-Maury L, Florencio FJ, Reyes JC. 2003. Arsenic sensing and resistance system in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *J. Bacteriol.* 185:5363–71
68. Luijten ML, de Weert J, Smidt H, Boschker HT, de Vos WM, et al. 2003. Description of *Sulfurospirillum balorespirans* sp. nov., and anaerobic, tetrachloroethene-respiring bacterium, and transfer of *Dehalospirillum multivorans* to the genus *Sulfurospirillum* as *Sulfurospirillum multivorans* comb. *Int. J. Syst. Evol. Microbiol.* 53:787–93
69. Macur RE, Jackson CR, Botero LM, McDermott TR, Inskip WP. 2004. Bacterial populations associated with the oxidation and reduction of arsenic in an unsaturated soil. *Environ. Sci. Technol.* 38:104–11
70. Macy JM, Nunan K, Hagen KD, Dixon DR, Harbour PJ, et al. 1996. *Chrysiogenes arsenatis* gen. nov., sp. nov., a new arsenate-respiring bacterium isolated from gold mine wastewater. *Int. J. Syst. Bacteriol.* 46:1153–57
71. Macy JM, Santini JM, Pauling BV, O'Neill AH, Sly LI. 2000. Two new arsenate-sulfate-reducing bacteria: mechanisms of arsenate reduction. *Arch. Microbiol.* 173:49–57
72. Maher MJ, Santini J, Pickering IJ, Prince RC, Macy JM, George GN. 2004. X-ray absorption spectroscopy of selenate reductase. *Inorg. Chem.* 43:402–4
73. Maki T, Hasegawa H, Watarai H, Ueda K. 2005. Classification for dimethylarsenate-decomposing bacteria using a restrict fragment length polymorphism analysis of 16S rRNA genes. *Anal. Sci.* 20:1–8
74. Malasarn D, Saltikov CW, Campbell KM, Santini JM, Hering JG, Newman DK. 2004. *arrA* is a reliable marker for As(V)-respiration in the environment. *Science* 306:455
75. Martin P, DeMel S, Shi J, Gladysheva T, Gatti DL, et al. 2001. Insights into the structure, solvation, and mechanism of ArsC arsenate reductase, a novel arsenic detoxification enzyme. *Structure* 9:1071–81
76. McBride BC, Wolfe RS. 1971. Biosynthesis of dimethylarsine by a methanobacterium. *Biochemistry* 10:4312–17
77. McCarty S, Chasteen T, Marshall M, Fall R, Bachofen R. 1993. Phototrophic bacteria produce volatile, methylated sulfur and selenium compounds. *FEMS Microbiol. Lett.* 112:93–98
78. McDevitt CA, Hanson GR, Noble CJ, Cheesman MR, McEwan AG. 2002. Characterization of the redox centers in dimethyl sulfide dehydrogenase from *Rhodovulum sulfidophilum*. *Biochemistry* 41:15234–44
79. McEwan AG, Ridge JP, McDevitt CA. 2002. The DMSO reductase family of microbial molybdenum enzymes; molecular properties and role in the dissimilatory reduction of toxic elements. *Geomicrobiol. J.* 9:3–21
80. Michalke K, Wickenheiser EB, Mehring M, Hirner AV, Hensel R. 2000. Production of volatile derivatives of metal(loid)s by microflora involved in anaerobic digestion of sewage sludge. *Appl. Environ. Microbiol.* 66:2791–96

81. Momplaisir GM, Rosal CG, Heithmar EM. 2001. Arsenic speciation methods for studying the environmental fate of organoarsenic animal-feed additives. U.S. EPA, NERL-Las Vegas, 2001. (TIM No. 01-11) <http://www.epa.gov/nerlesd1/chemistry/labmonitor/labresearch.htm>
82. Mukhopadhyay R, Rosen BP, Phung LT, Silver S. 2002. Microbial arsenic: from geocycles to genes and enzymes. *FEMS Microbiol. Rev.* 26:311-25
83. Muller D, Lievreumont D, Simeonova DD, Hubert JC, Lett MC. 2003. Arsenite oxidase *aox* genes from a metal-resistant β -proteobacterium. *J. Bacteriol.* 185:135-41
84. Muller S, Klein A. 2001. Coordinate positive regulation of genes encoding [NiFe] hydrogenases in *Methanococcus voltae*. *Mol. Genet. Genomics* 265:1069-75
85. Newman DK, Ahmann D, Morel FMM. 1998. A brief review of microbial arsenate respiration. *Geomicrobiol. J.* 15:255-68
86. Newman DK, Beveridge TJ, Morel FMM. 1997. Precipitation of As_2S_3 by *Desulfotomaculum auripigmentum*. *Appl. Environ. Microbiol.* 63:2022-28
87. Newman DK, Kennedy EK, Coates JD, Ahmann D, Ellis DJ, et al. 1997. Dissimilatory arsenate and sulfate reduction in *Desulfotomaculum auripigmentum* sp. nov. *Arch. Microbiol.* 168:380-88
88. Nickson RT, McArthur JM, Ravenscroft P, Burgess WG, Ahmed KM. 2000. Mechanism of arsenic release to groundwater, Bangladesh and West Bengal. *Appl. Geochem.* 15:403-13
89. Niggemyer A, Spring S, Stachebrandt E, Rosenzweig RF. 2001. Isolation and characterization of a novel As(V)-reducing bacterium: implications for arsenic mobilization and the genus *Desulfitobacterium*. *Appl. Environ. Microbiol.* 67:5568-80
90. Oremland RS, Dowdle PR, Hoeft S, Sharp JO, Schaefer JK, et al. 2000. Bacterial dissimilatory reduction of arsenate and sulfate in meromictic Mono Lake, California. *Geochem. Cosmochem. Acta* 64:3073-84
91. Oremland RS, Herbel MJ, Switzer Blum J, Langley S, Beveridge TJ, et al. 2004. Structural and spectral features of selenium nanospheres formed by Se-respiring bacteria. *Appl. Environ. Microbiol.* 70:52-60
- 92. Oremland RS, Hoeft SE, Santini JM, Bano N, Hollibaugh RA, Hollibaugh JT. 2002. Anaerobic oxidation of arsenite in Mono Lake water and by a facultative, arsenite-oxidizing chemoautotroph, strain MLHE-1. *Appl. Environ. Microbiol.* 68:4795-802**
93. Oremland RS, Hollibaugh JT, Maest AS, Presser TS, Miller L, Culbertson C. 1989. Selenate reduction to elemental selenium by anaerobic bacteria in sediments and culture: biogeochemical significance of a novel, sulfate-independent respiration. *Appl. Environ. Microbiol.* 55:2333-43
94. Oremland RS, Kulp TR, Switzer Blum J, Hoeft SE, Baesman S, et al. 2005. A microbial arsenic cycle in a salt-saturated, extreme environment: Searles Lake, California. *Science* 308:1305-8
95. Oremland RS, Steinber NA, Maest AS, Miller LG, Hollibaugh JT. 1990. Measurement of in situ rates of selenate removal by dissimilatory bacterial reduction in sediments. *Environ. Sci. Technol.* 24:1157-64
96. Oremland RS, Steinberg NA, Presser TA, Miller LG. 1991. In situ bacterial selenate reduction in the agricultural drainage systems of western Nevada. *Appl. Environ. Microbiol.* 57:615-17
97. Oremland RS, Stolz JF. 2003. The ecology of arsenic. *Science* 300:939-44
98. Oremland RS, Stolz JF. 2005. Arsenic, microbes and contaminated aquifers. *Trends Microbiol.* 13:45-49
99. Oremland RS, Stolz JF, Hollibaugh JT. 2004. The microbial arsenic cycle in Mono Lake, California. *FEMS Microbiol. Ecol.* 48:15-27

92. Described an organism that couples arsenite oxidation to dissimilatory nitrate reduction and proposed an anaerobic arsenic cycle.

100. Oremland RS, Zehr JP. 1986. Formation of methane and carbon dioxide from dimethylselenide in anoxic sediments and by a methanogenic bacterium. *Appl. Environ. Microbiol.* 52:1031–36
101. Pavkov M, Goessler W. 2004. Determination of organoarsenic compounds in finishing chicken feed and chicken litter by HPLC-ICP-MS. In *Arsenic Exposure and Health Effects IV*, ed. WR Chapell, CO Abernathy, RL Calderon, pp. 125–34. Oxford, UK: Elsevier
102. Perez-Jimenez JR, DeFraia C, Young LY. 2005. Arsenate respiratory gene (*arrA*) for *Desulfosporosinus* sp. strain Y5. *Biochem. Biophys. Res. Comm.* 338:825–29
103. Pinsent J. 1954. Need for selenite and molybdate in the formation of formic dehydrogenase by members of the *Escherichia coli*-*Aerobacter aerogenes* group of bacteria. *Biochem. J.* 57:10–16
104. Qin J, Rosen BP, Zhang Y, Wang G, Franke S, Rensing C. 2006. Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. *Proc. Natl. Acad. Sci. USA* 103:2075–80
105. Ranganathan M. 2005. *arr* genes from arsenate-respiring low G+C Gram positive bacteria *Bacillus selenitireducens* strain MLS10 and *Clostridium* sp. strain ObILAs. Masters thesis. Duquesne Univ.
106. Ranjard L, Nazaret Cournoyer B. 2003. Freshwater bacteria can methylate selenium through the thiopurine methyltransferase pathway. *Appl. Environ. Microbiol.* 69:3784–90
107. Ranjard L, Prigent-Combaret C, Favre-Bonte S, Monnez C, Nazaret S, Cournoyer B. 2004. Characterization of a novel selenium methyltransferase from freshwater bacteria showing strong similarities with the calicheamicin methyltransferase. *Biochem. Biophys. Acta* 1679:80–85
108. Ranjard L, Prigent-Combaret C, Nazaret S, Cournoyer B. 2002. Methylation of inorganic and organic selenium by the bacterial thiopurine methyltransferase. *J. Bacteriol.* 184:3146–49
109. Rhine ED, Garcia-Dominguez E, Phelps CD, Young LY. 2005. Environmental microbes can speciate and cycle arsenic. *Environ. Sci. Technol.* 39:9569–73
110. Rhine ED, Phelps CD, Young LY. 2006. Anaerobic arsenite oxidation by novel denitrifying isolates. *Environ. Microbiol.* 8:899–908
111. Ridley WP, Dizikes L, Cheh A, Wood JM. 1977. Recent studies on biomethylation and demethylation of toxic elements. *Environ. Health Perspect.* 19:43–46
112. Rosen BP. 2002. Biochemistry of arsenic detoxification. *FEBS Lett.* 529:86–92
113. Rother M, Mathes I, Lottspeich F, Böck A. 2003. Inactivation of the *selB* gene in *Methanococcus maripaludis*: effect on synthesis of selenoproteins and their sulfur-containing homologs. *J. Bacteriol.* 185:107–14
114. Rother M, Resch A, Gardner WL, Whitman WB, Böck A. 2001. Heterologous expression of archaeal selenoprotein genes directed by the SECIS element located in the 3' nontranslated region. *Mol. Microbiol.* 40:900–8
115. Rother M, Wilting R, Commans S, Böck A. 2000. Identification and characterization of the selenocysteine-specific translation factor SelB from the archaeon *Methanococcus jannaschii*. *J. Mol. Biol.* 299:351–58
116. Rutherford DW, Bednar AJ, Garbarino JR, Needham R, Staver KW, Wershaw RL. 2003. Environmental fate of roxarsone in poultry litter. II. Mobility of arsenic in soils amended with poultry litter. *Environ. Sci. Technol.* 37:1515–20
117. Salmassi TM, Venkateswaren K, Satmomi M, Nealson KH, Newman DK, Hering JG. 2002. Oxidation of arsenite by *Agrobacterium albertimagni*, AOL15, sp. nov. from Hot Creek, California. *Geomicrobiol. J.* 19:53–66

118. Saltikov CW, Cifuentes A, Venkateswaran K, Newman DK. 2002. 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.* 69:2800–9
119. Saltikov CW, Newman DK. 2003. Genetic identification of a respiratory arsenate reductase. *Proc. Natl. Acad. Sci. USA* 100:10983–88
120. Saltikov CW, Wildman RA, Newman DK. 2005. Expression dynamics of arsenic respiration and detoxification in *Shewanella* sp. strain ANA-3. *J. Bacteriol.* 187:7390–96
121. Santini JM, Sly LI, Schnagl RD, Macy JM. 2000. A new chemolithoautotrophic arsenite-oxidizing bacterium isolated from a gold mine: phylogenetic, physiological, and preliminary biochemical studies. *Appl. Environ. Microbiol.* 66:92–97
122. Santini JM, Sly LI, Wen A, Comrie D, de Wulf-Durand P, Macy JM. 2002. New arsenite-oxidizing bacteria isolated from Australian gold mining environments: phylogenetic relationships. *Geomicrobiol. J.* 19:67–76
123. Santini JM, Stolz JF. 2004. Prokaryote arsenate and selenate reduction. In *Strict and Facultative Anaerobes: Medical and Environmental Importance*, ed. MM Nakano, P Zuber, pp. 239–49. Norfolk, VA: Horizon Sci.
124. Santini JM, Stolz JF, Macy JM. 2002. Isolation of a new arsenate-respiring bacterium: physiological and phylogenetic studies. *Geomicrobiol. J.* 19:41–52
125. Santini JM, vanden Hoven RN. 2004. Molybdenum-containing arsenite oxidase of the chemolithoautotrophic arsenite oxidizer NT-26. *J. Bacteriol.* 186:1614–19
126. Sarathchandra SU, Watkinson JH. 1981. Oxidation of elemental selenium to selenite by *Bacillus megaterium*. *Science* 211:600–1
127. Sargent F, Berks BC, Palmer T. 2002. Assembly of membrane-bound respiratory complexes by the TAT protein-transport system. *Arch. Microbiol.* 178:77–84
128. Sayato Y, Nakamuro K, Hasegawa T. 1997. Selenium methylation and toxicity mechanism of selenocysteine. *Yakugaku Zasshi* 117:665–72
- 129. Schröder I, Rech S, Krafft T, Macy JM. 1997. Purification and characterization of the selenate reductase from *Tbaueria selenatis*. *J. Biol. Chem.* 272:23765–68**
130. Sehlin HM, Lindström BE. 1992. Oxidation and reduction of arsenic by *Sulfolobus acidocaldarius* strain BC. *FEMS Microbiol. Lett.* 93:87–92
131. Silver S, Phung LT. 2005. Genes and enzymes involved in the bacterial oxidation and reduction of inorganic arsenic. *App. Environ. Microbiol.* 71:599–608
132. Stadtman TC. 1996. Selenocysteine. *Annu. Rev. Biochem.* 65:83–100
133. Stolz JF, Basu P, Oremland RS. 2002. Microbial transformation of elements: the case of arsenic and selenium. *Int. Microbiol.* 5:201–7
134. Stolz JF, Ellis DJ, Switzer Blum J, Ahmann D, Lovley DR, Oremland RS. 1999. *Sulfurospirillum barnesii* sp. nov., *Sulfurospirillum arsenophilus* sp. nov., and the *Sulfurospirillum* clade in the epsilon proteobacteria. *Int. J. Syst. Bacteriol.* 49:1177–80
135. Stolz JF, Oremland RS. 1999. Bacterial respiration of arsenic and selenium. *FEMS Microbiol. Rev.* 23:615–27
136. Stupperich E. 1993. Recent advances in elucidation of biological corrinoid functions. *FEMS Microbiol. Rev.* 12:349–66
137. Switzer Blum J, Bindi AB, Buzzelli J, Stolz JF, Oremland RS. 1998. *Bacillus arsenosele-natis* sp. nov., and *Bacillus selenitireducens* sp. nov.: two haloalkaliphiles from Mono Lake, California, which respire oxyanions of selenium and arsenic. *Arch. Microbiol.* 171:19–30
138. Takai K, Hirayama H, Sakihama Y, Inagaki F, Yamato Y, Horikoshi K. 2002. Isolation and metabolic characteristics of previously uncultured members of the order *Aquificales* in a subsurface gold mine. *Appl. Environ. Microbiol.* 68:3046–54

129. Purification and characterization of the first respiratory selenate reductase.

139. Takai K, Kobayashi H, Nealson KH, Horikoshi K. 2003. *Deferribacter desulfuricans* sp. nov., a novel sulfur-, nitrate- and arsenate-reducing thermophile isolated from a deep-sea hydrothermal vent. *Int. J. Syst. Evol. Microbiol.* 53:839–46
140. Thomas DJ, Waters SB, Styblo M. 2004. Elucidating the pathway for arsenic methylation. *Toxicol. Appl. Pharmacol.* 198:319–26
141. Thorell HD, Stenklo K, Karlsson J, Nilsson T. 2003. A gene cluster for chlorate metabolism in *Ideonella dechloratans*. *Appl. Environ. Microbiol.* 69:5585–92
142. Turner RJ, Weiner JH, Taylor DE. 1998. Selenium metabolism in *Escherichia coli*. *Biometals* 11:223–27
143. vanden Hoven RN, Santini JM. 2004. Arsenite oxidation by the heterotroph *Hydrogenophaga* sp. str. NT-14: the arsenite oxidase and its physiological electron acceptor. *Biochem. Biophys. Acta* 1656:148–55
144. Xu C, Shi W, Rosen BP. 1996. The chromosomal *arsR* gene of *Escherichia coli* encodes a trans-acting metalloregulatory protein. *J. Biol. Chem.* 271:2427–32
145. Yang HC, Cheng J, Finan TM, Rosen BP, Bhattacharjee H. 2005. Novel pathway for arsenic detoxification in the legume symbiont *Sinorhizobium meliloti*. *J. Bacteriol.* 187:6991–97
146. Zobrist J, Dowdle PR, Davis JA, Oremland RS. 2000. Mobilization of arsenite by dissimilatory reduction of adsorbed arsenate. *Environ. Sci. Technol.* 34:4747–75

RELATED RESOURCES

- Aposhian HV. 1997. Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. *Annu. Rev. Pharmacol. Toxicol.* 37:397–419
- Silver S, Phung LT. 1996. Bacterial heavy metal resistance: New Surprises. *Annu. Rev. Microbiol.* 50:753–89
- Stadtman TC. 1990. Selenium biochemistry. *Annu. Rev. Biochem.* 59:111–27



Contents

Frontispiece <i>Dale Kaiser</i>	xvi
A Microbial Genetic Journey <i>Dale Kaiser</i>	1
Radical Enzymes in Anaerobes <i>Wolfgang Buckel and Bernard T. Golding</i>	27
The Structural and Functional Role of RNA in Icosahedral Virus Assembly <i>Anette Schneemann</i>	51
The Biology of the <i>Cryptococcus neoformans</i> Species Complex <i>Xiaorong Lin and Joseph Heitman</i>	69
Arsenic and Selenium in Microbial Metabolism <i>John F. Stolz, Partha Basu, Joanne M. Santini, and Ronald S. Oremland</i>	107
Curli Biogenesis and Function <i>Michelle M. Barnhart and Matthew R. Chapman</i>	131
Uranium Reduction <i>Judy D. Wall and Lee R. Krumboltz</i>	149
<i>Francisella tularensis</i> : Taxonomy, Genetics, and Immunopathogenesis of a Potential Agent of Biowarfare <i>Molly K. McLendon, Michael A. Apicella, and Lee-Ann H. Allen</i>	167
Manganese Transport and the Role of Manganese in Virulence <i>Krisztina M. Papp-Wallace and Michael E. Maguire</i>	187
Biochemical Aspects of Coronavirus Replication and Virus-Host Interaction <i>Luis Enjuanes, Fernando Almazán, Isabel Sola, and Sonia Zuñiga</i>	211
Environmental Stress and Lesion-Bypass DNA Polymerases <i>Takehiko Nobmi</i>	231

Environmental Biology of the Marine <i>Roseobacter</i> Lineage <i>Irene Wagner-Döbler and Hanno Biebl</i>	255
Defining Virulence Genes in the Dimorphic Fungi <i>Chad A. Rappleye and William E. Goldman</i>	281
Structure and Function of RNA Replication <i>Juan Ortín and Francisco Parra</i>	305
Streamlining and Simplification of Microbial Genome Architecture <i>Michael Lynch</i>	327
DnaA: Controlling the Initiation of Bacterial DNA Replication and More <i>Jon M. Kaguni</i>	351
The Bacterial Twin-Arginine Translocation Pathway <i>Philip A. Lee, Danielle Tullman-Ercek, and George Georgiou</i>	373
Surface Proteins of Gram-Positive Bacteria and How They Get There <i>June R. Scott and Timothy C. Barnett</i>	397
Subterfuge and Manipulation: Type III Effector Proteins of Phytopathogenic Bacteria <i>Sarah R. Grant, Emily J. Fisher, Jeff H. Chang, Beth M. Mole, and Jeffery L. Dangl</i>	425
Induction of Competence Regulons as a General Response to Stress in Gram-Positive Bacteria <i>Jean-Pierre Claverys, Marc Prudhomme, and Bernard Martin</i>	451
Origin of Mutations Under Selection: The Adaptive Mutation Controversy <i>John R. Roth, Elisabeth Kugelberg, Andrew B. Reams, Eric Kofoid, and Dan I. Andersson</i>	477
Virus Counterdefense: Diverse Strategies for Evading the RNA-Silencing Immunity <i>Feng Li and Shou-Wei Ding</i>	503
Understanding Microbial Metabolism <i>Diana M. Downs</i>	533
Multilocus Sequence Typing of Bacteria <i>Martin C.J. Maiden</i>	561