Chapter 6
Bacteria Versus Selenium: A View from the Inside Out

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Abstract Bacteria and selenium (Se) are closely interlinked as the element serves both essential nutrient requirements and energy generation functions. However, Se can also behave as a powerful toxicant for bacterial homeostasis. Conversely, bacteria play a tremendous role in the cycling of Se between different environmental compartments, and bacterial metabolism has been shown to participate to all valence state transformations undergone by Se in nature. Bacteria possess an extensive molecular repertoire for Se metabolism. At the end of the 1980s, a novel mode of anaerobic respiration based on Se oxyanions was experimentally documented for the first time. Following this discovery, specific enzymes capable of reducing Se oxyanions and harvesting energy were found in a number of anaerobic bacteria. The genes involved in the expression of these enzymes have later been identified and cloned. This iterative approach undertaken *outside-in* led to the understanding of the molecular mechanisms of Se transformations in bacteria. Based on the extensive knowledge accumulated over the years, we now have a full(er) view from the *inside out*, from DNA-encoding genes to enzymes and thermodynamics. Bacterial transformations of Se for assimilatory purposes have been the object of numerous studies predating the investigation of Se respiration. Remarkable contributions related to the understating of the molecular picture underlying seleno-amino acid biosynthesis are reviewed herein. Under certain circumstances, Se is a toxicant for bacterial metabolism and bacteria have evolved strategies to counteract this toxicity, most notably by the formation of elemental Se (nano)particles. Several biotechnological

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applications, such as the production of functional materials and the biofortification of crop species using Se-utilizing bacteria, are presented in this chapter.

Keywords Selenium • Bacteria • Anaerobic respiration • Selenium detoxification • Selenoenzymes

6.1 Introduction

Bacteria are involved in the cycling of selenium (Se) through different compartments of the environment. Se has several oxidation states: (VI), (IV), (0), and (-II), that display variable solubility, bioavailability, and toxicological profiles (Chapman et al. 2010). Se oxyanions, SeO_x, i.e. selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), are water-soluble, bioavailable, and toxic to aquatic life (Simmons and Wallschlaeger 2005). Elemental Se (Se^0) is considered practically nontoxic in view of its solid state and negligible water solubility. However, a number of reports documented adverse effects exhibited by Se^0 against ecological receptors such as filter-feeding mollusks and fish (Chapman et al. 2010). The bioremediation approach employed by various bioreactor systems relies on the microbial conversion of SeO_x to Se^0 (Staicu et al. 2015a). Selenide, Se(−II), the most reduced valence state of Se, is present in strongly reducing conditions. Both inorganic Se, e.g. hydrogen selenide (H_2Se and HSe^-), metal selenides ($M^{n+}Se^{2-}$), and selenocyanate ($SeCN^-$), and organic selenides (e.g. methylated species such as dimethylselenide (DMSe), aminoacids such as selenocysteine (Sec or SeCys) and selenomethionine (SeMet), and metabolic products (e.g. trimethylselenonium) have been described (Fernandez-Martinez and Charlet 2009).

A biogeochemical cycle of inorganic and organic forms of Se was first proposed in a seminal article by Shrift (1964). Following this article, bacteria were later found to participate in most transformations undergone by Se in aquatic and terrestrial ecosystems. A major finding that came out at the end of 1980s was the capacity of some anaerobic bacteria to use Se as terminal electron acceptor for cellular respiration (Macy et al. 1989; Oremland et al. 1989). The first bacteria described that carry out anaerobic respiration on selenate were *Thauera selenatis*, belonging to the beta subclass (Macy et al. 1993), and *Sulfurospirillum barnesii* and *S. arsenophilus* of the epsilon subclass of Proteobacteria (Oremland et al. 1994; Laverman et al. 1995; Stolz and Oremland 1999). *T. selenatis* was isolated in California by Joan Macy and coworkers from a bioreactor setting treating agricultural wastewater rich in Se-oxyanion effluents. *S. barnesii* was isolated from a Se-rich drainage slough located near Fallon, NV (Oremland et al. 1994). Apart from their use in cellular respiration, Se compounds can also behave as powerful toxicants due to the production of dysfunctional biomolecules when Se is mis-incorporated into sulfur-rich proteins (Stadtman 1974). Additionally, the metabolism of SeO_x was linked to oxidative stress (Hoffman 2002). However, bacteria have evolved different strategies

to counteract this toxicity and a major one is the production of Se^0 nanoparticles with significantly reduced toxicity.

This chapter discusses the bacterial transformations of Se from a molecular biology perspective. We briefly examine the main strategies employed by anaerobic and aerobic bacteria to transform Se, and then we expound upon the genetics and the enzymes that underlie these transformations. The chapter also presents the potential use of Se-transforming bacteria for the production of functional materials and the biofortification of plant and crop species. The reader is referred to some recent reviews for further details on this re-emerging subject of scientific interest (Santos et al. 2015; Winkel et al. 2015).

6.2 Bacterial Metabolism of Selenium

6.2.1 Selenium Respiration

Under anaerobic conditions, various electron acceptors (e.g. NO_3^-, SO_4^{2-}, S^0, Fe^{3+} or Mn^{4+}) can be utilized by bacteria for respiration as the terminal step of their electron transport chain. Macy et al. (1989) showed that selenate can be used by bacteria for cellular respiration. This strategy is termed *dissimilatory* reduction. From a thermodynamic point of view, the reduction of selenate to selenite coupled with the oxidation of an electron donor, such as formate, acetate (Eq. 6.1) or lactate (Eq. 6.2) provides energy to support bacterial growth (Macy et al. 1989; Oremland et al. 1994):

$$C_2H_3O_2^- + 4SeO_4^{2} + H^+ \rightarrow 4SeO_3^{2} + 2CO_2 + 2H_2O \tag{6.1}$$

$$\Delta G_f \circ = -556kJ\ /\ mol\ \text{acetate}\ (C_2H_3O_2^-)$$

$$C_3H_6O_3^- + 2SeO_4^{2} \rightarrow 2SeO_3^{2} + C_2H_3O_2^- + HCO_3^- + H^+ \tag{6.2}$$

$$\Delta G_f \circ = -343.1kJ\ /\ mol\ \text{lactate}\ (C_3H_6O_3^-)$$

Provided the bacteria involved are capable of metabolizing both selenate and selenite, usually sequentially, then elemental Se is the end product of selenate reduction according to Eq. 6.3 (Oremland et al. 1994):

$$3C_3H_6O_3^- + 2SeO_4^{2} + H^+ \rightarrow 2Se^0 + 3C_2H_3O_2^- + 3HCO_3^- + 2H_2O \tag{6.3}$$

$$\Delta G_f \circ = -467.4kJ\ /\ mol\ \text{lactate}$$

Some species can respire either selenate or selenite, but not both. For example, *Bacillus selenitireducens*, a halo-alkaliphile isolated from Mono Lake, California,

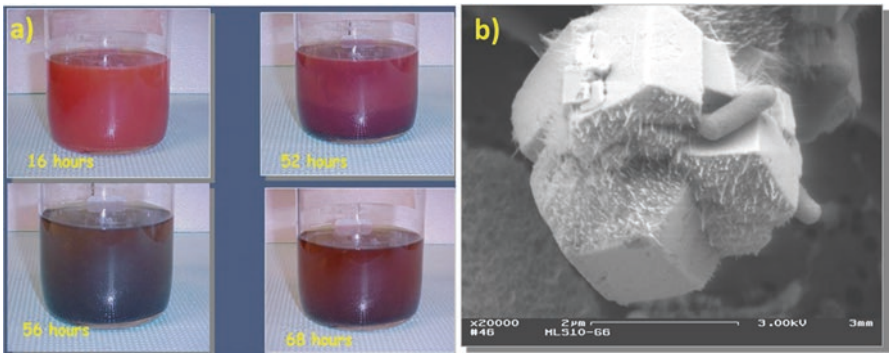

Fig. 6.1 Biogenic Se^0 and HSe^-: (**a**) Formation of HSe^- from accumulated Se^0 over a time sequence by *B. selenitireducens* incubated with an excess of the electron donor lactate. The black precipitate at the bottom of the bottle was the hexagonal crystalline allotrope of Se^0, while the red/orange was the amorphous/monoclinic allotrope of Se^0 (Photos courtesy of M. Herbel); (**b**) Scanning electron micrograph of a black hexagonal crystal of Se^0 taken at the end of the above time course. The hair-like threads on the crystal consist of Se^0 formed by auto-oxidation of HSe^- when exposed to air (M. Herbel, unpublished)

can carry out the reductive dissimilation of selenite (Eq. 6.4) that yields energy for growth (Switzer Blum et al. 1998):

$$C_3H_6O_3^- + SeO_3^{2} + H^+ \rightarrow Se^0 + C_2H_3O_2^- + HCO_3^- + H_2O \qquad (6.4)$$

$$\Delta G_f \circ = -241.6 kJ / mol \text{ lactate} (pH = 10)$$

If provided with an excess of electron donor (lactate) over the available selenite supplied, then this microorganism can carry out a further reduction of the accumulated mass of extracellular Se^0 to HSe^-, according to Eq. 6.5 (Herbel et al. 2003):

$$C_3H_6O_3^- + 2Se^0 + 2H_2O \rightarrow 2HSe^- + C_2H_3O_2^- + HCO_3^- + 3H^+ \qquad (6.5)$$

$$\Delta G_f \circ = -98.8 kJ / mol \text{ lactate} (pH = 10)$$

The time course progression of this reaction is visually quite striking. It starts out with the mass accumulation of bright orange amorphous Se^0, which grows darker and separates into layers, and eventually clears into a tawny-tinged fluid with a dense, black precipitate of hexagonal Se^0 at the bottom of the large serum bottle (Fig. 6.1a). If one removes the rubber stopper allowing air to exchange with the N_2 headspace, a rapid exothermic reaction occurs whereby the accumulated HSe^- autoxidizes back to Se^0, and the bottle turns back to the thick orange color. A scanning electron micrograph of the black hexagonal Se^0 allotrope is shown in Fig. 1b, where the thread-like hairs seen on its surface are strands of Se^0 formed by the oxidation of HSe^- upon its exposure to the oxygen in air. A cell of *B. selenitireducens* also

adorns the surface of the crystal. Se respiration in Archaea was only marginally reported to date (Huber et al. 2000).

6.2.2 Selenium Assimilation

In contrast to the dissimilatory reduction of SeO_x used to energize the bacterial cell, the *assimilatory* reduction of Se oxyanions is employed by both aerobes and anaerobes for the synthesis of Se amino acids, namely Sec and SeMet. These amino acids are incorporated into selenoproteins having essential roles in the proper functioning of bacterial metabolism. In selenoproteins, Se has structural and enzymatic roles, serving oxidoreductase functions against reactive oxygen species (ROS) (Labunskyy et al. 2014). Sects. 6.3.3, 6.3.4, 6.3.5 from this chapter provide an in-depth presentation of the seleno-amino acids metabolism.

6.2.3 Selenium Detoxification

Se exerts toxic effects on bacteria and several mechanisms have been proposed for the reduction of selenite to Se^0 in microorganisms, including a glutathione (GSH) system, thioredoxin system, siderophore-mediated reduction, sulfide-mediated reduction, and dissimilatory reduction (Zannoni et al. 2008).

The reduction sequence of selenite to Se^0 by GSH occurs according to the following reactions (Eqs. 6.6, 6.7, 6.8, 6.9, and 6.10) (Ganther 1968):

$$H_2SeO_3 + GSH \rightarrow GSSeO_2H + H_2O \tag{6.6}$$

$$GSSeO_2H + GSH \rightarrow GSSeOSG + H_2O \tag{6.7}$$

$$GSSeOSG + GSH \rightarrow GS - Se - SG + GSOH \tag{6.8}$$

$$GS–Se–SG + NADPH + H^+ \rightarrow GS–SeH + GSH + NADP^+ \tag{6.9}$$

$$GSSeH + GSH \rightarrow GSH + Se^0 \tag{6.10}$$

where $GSSeO_2H$, glutathione selenone; GSSeOSG, diglutathione selenone; GS-Se-SG, selenodiglutathione; and GSSeH, L-γ-glutamyl-*S*-selanyl-L-cysteinylglycine.

Reduced thioredoxin and thioredoxin reductase were hypothesized to be involved in the reduction of selenite and GS-Se-SG (Björnstedt et al. 1992). Reduced thioredoxin reacts with GS-Se-SG to form oxidized thioredoxin, reduced GSH, and selenopersulfide anion, and then Se^0 is released from the reactive selenopersulfide anion. In addition, selenite can react with the reactive biogenic sulfide abiotically, yielding Se^0 and elemental sulfur, S^0 (Hockin and Gadd 2003; Pettine et al. 2013). An iron siderophore, pyridine-2,6-bis(thiocarboxylic acid), produced by

Pseudomonas stutzeri KC, has also been proposed to detoxify selenite through reduction and formation of insoluble Se^0 precipitates (Zawadzka et al. 2006).

The exposure of bacteria to growth media containing selenite resulted in phenotypic changes and altered cell morphology in *Wolinella succinogenes* (Tomei et al. 1992) and *Desulfovibrio desulfuricans* DSM 1924 (Tomei et al. 1995). *P. moraviensis* stanleyae showed impaired growth (40% less bacterial cell density during stationary phase) and extended lag time when it was exposed to 10 mM sodium selenite (Staicu et al. 2015b). Figure 6.2 presents Se^0 nanoparticles with a diameter around 60 nm that were found intracellularly, following exposure of *P. moraviensis* stanleyae to sodium selenite. Additionally, the Se^0 enzyme assay identified GSH, nitrite, and sulfite reductases as candidate enzymes involved in selenite reduction, suggestive of a detoxification mechanism at play (Ni et al. 2015). Aerobic reduction of selenite is ubiquitous amongst phylogenetically diverse bacterial groups, indicating shared metabolic pathways used for the reduction of other oxyanions such as nitrate or sulfate (Sura-de Jong et al. 2015).

6.2.4 Metabolic Explorations Using Se Isotopic Techniques

Further detailed investigations into the actual scope and rates of Se biotransformations occurring within Se-impacted and pristine environments were facilitated by the use of both radioisotopes (i.e., ^{75}Se-selenate) and the fact that this element also displays six naturally-occurring stable isotopes (i.e. ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, and ^{80}Se). A radioisotopic procedure was devised whereby ^{75}Se-selenate was injected into subcores recovered from anoxic sediments, and after incubation and washing, the amount of $^{75}Se^0$ was quantified so as to yield rate constants (Oremland et al. 1990). Multiplication of the rate constants by the concentration of selenate in pore waters ($\leq$ 40 μM) yielded *in situ* rates of dissimilatory selenate reduction. The rates determined for a large agricultural evaporation pond located in the Se-impacted San Joaquin Valley (California) were calculated to be 300 μM SeO_4^{2-} per m^2 per day, which was sufficient to sequester all the pond water Se oxyanions as Se^0 in the bottom sediments within ~90 days. Similar results were obtained for an agricultural drainage slough located in western Nevada (Oremland et al. 1991). To answer the question of whether or not this phenomenon was widespread or confined to Se-contaminated regions, a broad survey was conducted to assay surficial aquatic sediments from a number of different locales and chemistries (e.g. freshwater, estuarine, soda lakes, contaminated ponds, and saturated salterns). The results were surprising in that rapid dissimilatory selenate reduction was found to be common to all of the 11 sediment types investigated, indicating that both the bacteria involved and their enzymes were constitutive and active in these diverse biomes (Steinberg and Oremland 1990). In stark contrast to these rapid Se(VI) reduction rates, the rates of oxidation of $^{75}Se^0$ back to soluble oxyanions by bacterial cultures, as well as

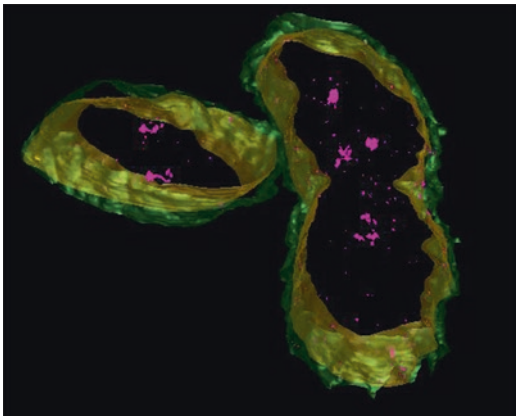

Fig. 6.2 Electron tomographic reconstruction with osmium staining of *P. moraviensis* stanleyae exposed to 10 mM of sodium selenite under aerobic conditions. Legend: The outer membrane (*in green*), inner membrane (*in yellow*), and Se^0 nanoparticles (*in pink*) (Adapted from Ni et al. 2015)

by oxic sediments, were very slow, with turnover rates measured in years rather than hours or days (Dowdle and Oremland 1998).

Selenate- and selenite-respiring bacteria are capable of a "classic" biological stable isotopic fractionation, selecting for the lighter isotopes, while leaving behind the heavier, as was demonstrated with pure cultures (Johnson et al. 1999). Cumulative reduction of Se(VI) and Se(IV) resulted in an enrichment of the $\delta^{80/76}$Se of ~11 per mil (Herbel et al. 2000). However, although fractionation was observed during incubation of live, manipulated sediment slurries (Ellis et al. 2003), little if any fractionation was observed in Se-contaminated drainages (Herbel et al. 2002). The absence of fractionation *in situ* was probably owing to the rapidity and completeness of the biological reactions involved. Nonetheless, Zhu et al. (2014) reported that at one particular site of an exposed outcrop in China, very broad ranges in the $\delta^{82/76}$Se ratios were observed (−14.2 to +11.37 per mil) reflecting on the alternating seasonal oxic vs. anoxic conditions in this subsurface aquifer. Analytical advances have been made which explore the use of stable oxygen isotopes ($^{18}O/^{16}O$) in Se oxyanions during dissimilatory reduction (Schellenger et al. 2015) which opens the possibility in the near future of employing multiple isotopes of Se and O (i.e. "clumped analyses") to better characterize the biogeochemical redox cycle of Se in nature.

6.3 Genes and Enzymes Involved in Bacterial Selenium Metabolism

6.3.1 Overview

As described above, inorganic and organic forms of Se are metabolized in a dissimilatory or assimilatory manner by Se-utilizing bacteria (Fig. 6.3). Some bacteria can use the Se oxyanions, selenate and selenite, as electron acceptors under anaerobic conditions, and other species can use these ions as substrates for producing

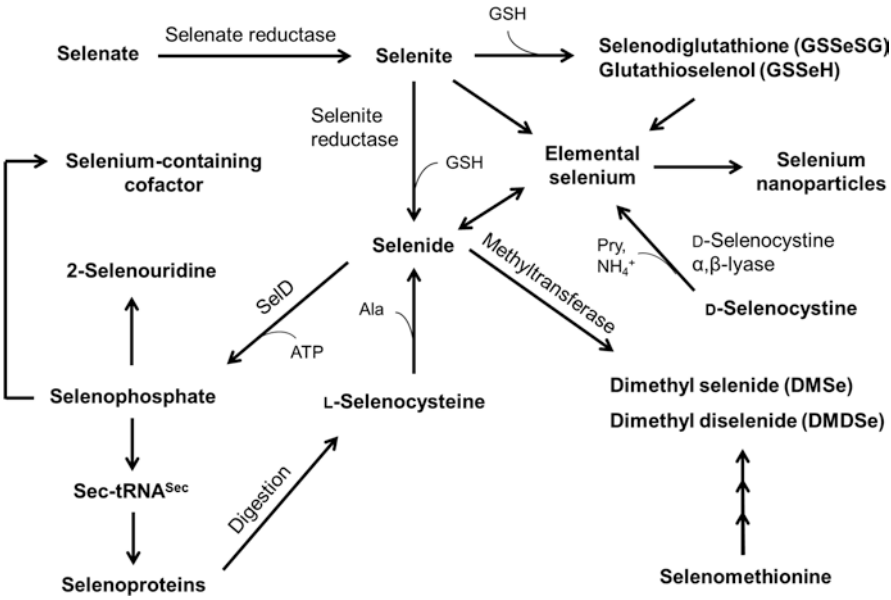

Fig. 6.3 Selenium metabolism in bacteria

biologically active Se compounds such as seleno-amino acids, selenouridine (SeU), and Se-containing cofactors. Until recently, only a few bacterial genes involved in Se utilization had been identified. Due to improvements in genome sequencing technology, genomic analysis has become a powerful way to predict if a bacterium can utilize Se. Previous studies identified genes involved in a specific Se-utilizing system. Based on the results of genomic screens using those already-identified genes as indicators, an increased number of Se-utilizing bacteria have been recognized.

Studies on the metabolism of Se oxyanions have identified genes responsible for selenate reduction, like *serABCD* in *Thauera selenatis* (Lowe et al. 2010), *srdBCA* in *Bacillus selenatarsenatis* SF-1 (Kuroda et al. 2011), and the *ygfKLMN* and *ynfEGHdmsD* operons in *Escherichia coli* (Bebien et al. 2002; Guymer et al. 2009). The microbial selenite reduction processes can be categorized broadly into either detoxification or anaerobic respiration. However, only a few selenite-respiring bacteria have been isolated (Stolz et al. 2006), and specific genes involved in selenite reduction have not as yet been identified. Various mechanisms have been proposed for the reduction of selenite to elemental Se, including the Painter-type reaction, a thioredoxin reductase system, siderophore-mediated reduction, sulfide-mediated reduction, and dissimilatory reduction (Zannoni et al. 2008).

Metabolic processes of biologically active Se molecules, such as seleno-amino acids, SeU, and Se-containing cofactors, have been characterized and their related genes have been identified. Such genes identified so far include those for biosynthesis of Sec (*selA*, *selB*, *selC*, and *selD* in bacteria and *PSTK*, *SepSecS*, *selB*, *selC*, and

selD in archaea), 2-SeU (*ybbB*), and Se-containing cofactors (*yqeB* and *yqeC*). Since the *selD* gene encoding selenophosphate synthetase (SelD) is commonly required for the synthesis of Sec, SeU, and Se-containing cofactors, it is one of the major biomarkers for identifying bacterial species that can utilize Se (Zhang et al. 2006; Lin et al. 2015). Recently, Peng et al. (2016) applied comparative genomic approaches to more than 5200 sequenced bacterial genomes to investigate Se utilization in bacteria. Among the species examined, 1121 Sec-utilizing (21.5%), 980 SeU-utilizing (18.8%) and 312 Se-cofactor-utilizing (6.0%) organisms were identified. Se utilization is hypothesized to be an ancient trait that was once common to almost all bacterial species. However, this study only detected the presence of *selD* in 1754 organisms (33.7%), suggesting that most species have lost the ability to use Se over the long process of evolution.

6.3.2 Metabolism of Selenium Oxyanions

Bacteria interact with all valence states of Se, thus contributing to the biogeochemical cycle of this element (Shrift 1964). However, the reduction of high-valence states of Se is more often reported and occurs at a considerably faster pace than the oxidative side of the cycle.

6.3.2.1 Selenate Respiration and Reduction

As shown in Eq. 6.11 and 6.12, selenate reduction occurs by a two-step process. Selenate reduction to selenite is catalyzed by selenate reductases, and then selenite reduction to insoluble Se^0 is catalyzed by nonspecific selenite reductases. Such reductions can be observed under aerobic, anoxic, and anaerobic conditions. The genes and enzymes involved in selenate reduction were investigated especially in *Thauera selenatis*, *Enterobacter cloacae* SLD1a-1, *E. coli*, and *Bacillus selenatarsenatis* SF-1.

$$SeO_4^{2} + 2e + 2H^{+} \rightarrow SeO_3^{2} + H_2O \tag{6.11}$$

$$SeO_3^{2} + 4e + 6H^{+} \rightarrow Se^0 + 3H_2O \tag{6.12}$$

The Gram-negative bacterium *T. selenatis* can effectively reduce selenate to selenite anaerobically (Rech and Macy 1992). The selenate to selenite reduction reaction occurs in the periplasmic compartment. The first identified respiratory selenate reductase of the bacterium was purified and characterized (Schröder et al. 1997). This enzyme consists of a catalytic unit (SerA), an Fe-S protein (SerB), a heme *b* protein (SerC), and a molybdenum cofactor (Lowe et al. 2010). Complete inhibition of selenate reduction was achieved in the presence of both myxothiazol and

2-*n*-heptyl-4-hydroxyquinoline *N*-oxide, suggesting the involvement of both a quinol cytochrome *c* reductase and a quinol dehydrogenase in selenate reduction (Lowe et al. 2010). In addition, a novel 95-kDa protein, SefA (Se factor A), was isolated from the elemental Se secreted from *T. selenatis* cells into the extracellular medium, suggesting that the SefA protein aids in the secretion process by stabilizing Se nanospheres and preventing their aggregation (Debieux et al. 2011).

Enterobacter cloacae SLD1a-1 is a selenate-reducing bacterium isolated from the Se-rich waters of the San Luis Drain in California (Losi and Frankenberger 1997). Selenate reductase of *E. cloacae* SLD1a-1 is a membrane-bound trimeric complex with a catalytic subunit of 100 kDa, which may contain molybdenum as a cofactor (Ridley et al. 2006). In *E. coli*, at least three systems for selenate reduction have been identified. Selenate reductase encoded within the *ynfEGHdmsD* operon is dependent on the twin arginine translocation (Tat) system (Guymer et al. 2009). The catalytic subunit YnfE is predicted to bind a bis-molybdopterin guanine dinucleotide cofactor and a [4Fe-4S] cluster. The small subunit YnfG exhibits four [4Fe-4S]-binding motifs, with each motif containing four conserved cysteine residues. On the other hand, it has been demonstrated using gene deletion analyses that another *E. coli* selenate reductase is a structural complex including the proteins YgfK, YgfM, and YgfN, encoded by the *ygfKLMN* putative operon (Bebien et al. 2002). Although the specific activity is low, *E. coli* nitrate reductases A and Z (encoded by *narGHIJ* and *narZUWV*, respectively) and periplasmic nitrate reductase NapA also possess selenate reductase activity (Avazeri et al. 1997).

A Gram-positive bacterium, *B. selenatarsenatis* SF-1, was also isolated as a selenate-reducing bacterium (Fujita et al. 1997). The strain shows a stoichiometric relationship between cell growth, lactate consumption, and selenate reduction. It was demonstrated using transposon mutagenesis that the *srdBCA* operon encodes a putative oxidoreductase complex as a respiratory selenate reductase complex (Kuroda et al. 2011). The selenate reductase SrdBCA is a membrane-bound, trimeric molybdoenzyme. Electrons from the quinol pool are channeled to the catalytic subunit SrdA via SrdB, an Fe-S protein, and selenate receives the electrons from SrdA via the molybdenum cofactor (Kuroda et al. 2011).

6.3.2.2 Selenite and Elemental Selenium Respiration and Reduction

Certain selenate-reducing bacteria can also perform dissimilatory selenite reduction (Nancharaiah and Lens 2015). However, the investigation of selenite reduction via respiratory electron transport pathways is limited to a study using *Shewanella oneidensis* MR-1 (Li et al. 2014). Apart from respiration, selenite can also be reduced by bacteria as a dissimilatory strategy which includes detoxification.

To date, there are only a few studies on microbial reduction of Se^0 to selenide. Some of selenate- or selenite-respiring bacteria may have the capacity to reduce Se^0 as well. *Bacillus selenitireducens*, a selenite-respiring bacterium, produced significant amounts of selenide from Se^0 or selenite (Herbel et al. 2003). However, the reduction of Se^0 to selenide was not observed in the case of selenate-respiring bacteria, and the responsible catalytic enzymes have not been identified.

6.3.2.3 Selenium Nanoparticles

Many bacteria synthesize Se nanoparticles (SeNPs) as a mechanism of Se detoxification (Kessi et al. 1999). SeNPs have wide applications in medicine, therapeutics, biosensors, and environmental remediation (Wadhwani et al. 2016). Synthesis of these nanoparticles can be extracellular, intracellular, or membrane-bound. Se deposits were first observed on cell walls and cell membranes of *E. coli* under electron microscopy (Gerrard et al. 1974). After that, several bacterial species, both Gram-negative and Gram-positive, like *Veillonella atypica* and *Pseudomonas* sp. RB, have been demonstrated to synthesize quantum dots such as CdSe and ZnSe (Pearce et al. 2008; Ayano et al. 2014). After Se reduction based on the previously mentioned mechanisms, SeNPs accumulate in bacterial cells during mid- to late-exponential growth phases and are secreted into the surrounding medium in the stationary phase (Butler et al. 2012). Se factor A (SefA), a protein of approximately 95 kDa, accompanies SeNPs during their export from the cytoplasmic compartment, and aids in biomineralization and stabilization of the nanoparticles (Butler et al. 2012). Furthermore, the metalloid reductase RarA has the highest number of peptides with strong affinity for SeNPs, thereby conferring stability (Lenz et al. 2011). A reductase enzyme is responsible for the conversion of selenate and selenite to nano-Se in bacteria, and this phenomenon has mostly been studied in *T. selenatis*, *E. coli*, and *E. cloacae* (Wadhwani et al. 2016). However, the existence of multiple electron transport pathways has not been ruled out, and the mechanism by which these nanoparticles exhibit antimicrobial action is still unclear.

6.3.3 Selenoprotein Biosynthesis in Bacteria and Archaea

6.3.3.1 Selenocysteine Synthesis

The most important and best-characterized biological form of Se is the amino acid Sec. Most studies on selenoprotein biosynthesis in bacteria have been carried out with *E. coli*. Böck et al. revealed that at least four Sec-specific genes, *selA, selB, selC*, and *selD*, are required for bacterial Sec synthesis (Fig. 6.4) (Forchhammer et al. 1990; Leinfelder et al. 1990; Forchhammer et al. 1991). The gene *selC* encodes the Sec-specific tRNA ($tRNA^{Sec}$) and its anticodon UCA is complementary to the Sec codon UGA (Leinfelder et al. 1989). This tRNA is the largest tRNA in *E. coli* and has a unique modification pattern (Schon et al. 1989). However, the most obvious distinction between $tRNA^{Sec}$ and canonical elongator tRNAs is the eight-base-pair aminoacyl-acceptor stem; all other tRNA species have a seven base-pair stem. $tRNA^{Sec}$ is first aminoacylated with L-serine by seryl-tRNA synthetase and then the conversion of seryl-$tRNA^{Sec}$ into selenocysteyl-$tRNA^{Sec}$ (Sec-$tRNA^{Sec}$) is catalyzed by Sec synthase (the *selA* gene product) using selenophosphate. The Sec synthase SelA of *E. coli* binds pyridoxal 5′-phosphate (PLP) as a cofactor, the carbonyl of which forms an aldimine linkage with serine's α-amino group, and 2,3-elimination

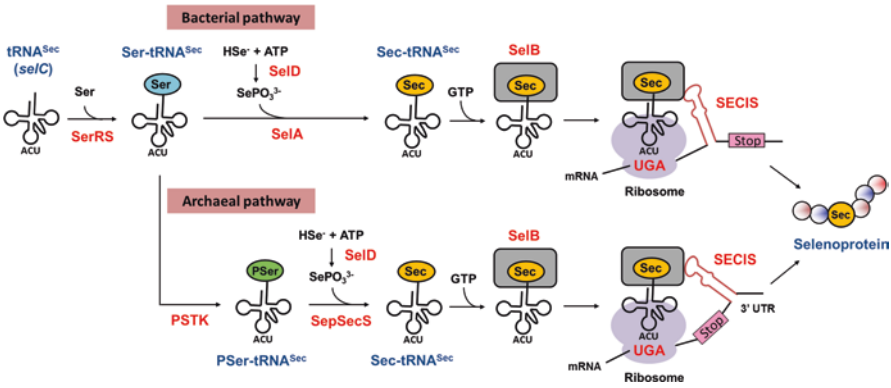

Fig. 6.4 Selenoprotein biosynthesis in bacteria and archaea. SerRS, seryl-tRNA synthetase; SelA, selenocysteine synthase; SelD, selenophosphate synthetase; GTP, guanosine-5′-triphosphate; SECIS, selenocysteine insertion sequence; SelB, SECIS-binding protein; PSKT, *O*-phosphoseryl-tRNA^{Sec} kinase; SepSecS, *O*-phosphoseryl-tRNA^{Sec}: selenocysteine synthase

of a water molecule generates enzyme-bound aminoacrylyl-tRNA^{Sec} (Forchhammer and Böck 1991). Nucleophilic addition of selenide to the aminoacrylyl double bond forms Sec-tRNA^{Sec}. The activated Se donor for the reaction, selenophosphate, is synthesized by selenophosphate synthetase, SelD (the product of the *selD* gene) (Veres et al. 1992). Due to the high K_m value of SelD for selenide (HSe^-), it is assumed that selenide is not the true substrate, but rather some activated, protein-bound Se species may specifically supply a substrate to selenophosphate synthetase.

Archaeal Sec-tRNA^{Sec} biosynthesis has some differences from the bacterial system (Fig. 6.4). Archaea and bacteria use a similar Sec-tRNA^{Sec} biosynthesis system, but archaea require an additional step, phosphorylation of Ser-tRNASec by *O*-phosphoseryl-tRNA^{Sec} kinase (PSTK) (Yuan et al. 2006). Investigating the process of Sec synthesis and incorporation in archaea started with the genome sequence of *Methanococcus jannaschii* (Bult et al. 1996). This genome encodes tRNA^{Sec} and its predicted structure resembles eukaryotic tRNA^{Sec} more closely than bacterial tRNA^{Sec} (Commans and Böck 1999). *In vitro* characterization of a *M. maripaludis* homolog of the eukaryotic *O*-phosphoseryl-tRNA^{Sec}:Sec synthase (SepSecS) showed its ability to catalyze the selenophosphate-dependent conversion of *O*-phosphoseryl-tRNA^{Sec} to Sec-tRNA^{Sec} (Yuan et al. 2006). Briefly, in the case of archaeal Sec-tRNA^{Sec} biosynthesis, after charging tRNA^{Sec} with serine, *O*-phosphoseryl-tRNA^{Sec} is synthesized as an intermediate upon phosphorylation of the seryl-tRNA^{Sec} by PSTK. Ultimately, *O*-phosphoseryl-tRNA^{Sec} is converted to Sec-tRNA^{Sec} by SepSecS using selenophosphate as the Se donor.

6.3.3.2 Selenocysteine Insertion

Because of its unique structural features, $tRNA^{Sec}$ is not recognized by the canonical elongation factor EF-Tu (Forster et al. 1990). Instead, a Sec-specific translation elongation factor, SelB, the product of the *selB* gene, is utilized for Sec insertion into a nascent polypeptide (Forchhammer et al. 1989). The N-terminus of SelB from *E. coli* shares significant homology to EF-Tu and it binds Sec-$tRNA^{Sec}$ and GTP stoichiometrically (Baron and Böck 1991). A unique property of bacterial SelB, crucial for its function, is its interaction with the selenoprotein mRNA. The segment responsible for this interaction, the SECIS element, has a stem-loop structure of approximately 40 nucleotides located immediately downstream of the UGA codon. Formation of the quaternary complex of SelB, Sec-$tRNA^{Sec}$, the SECIS, and GTP is cooperative and promotes Sec insertion into bacterial selenoproteins (Fig. 6.4). In addition, both *selA* and *selC* from the Gram-positive bacterium *Moorella thermoacetica* complement the corresponding genes in *E. coli* (Tormay et al. 1994; Kromayer et al. 1996). Thus, Gram-positive bacteria appear to utilize the same general strategy for Sec synthesis as Gram-negative bacteria.

In archaea, the Sec insertion system is still not fully proven. Analysis of Sec-coding genes in *M. voltae* first showed that Sec insertion in archaea is also directed by UGA (Halboth and Klein 1992). However, archaea do not have conserved SECIS sequences within the coding region of selenoprotein mRNAs (Fig. 6.4). Instead, conserved hairpin structures for different selenoprotein mRNAs were only found in untranslated regions of *M. jannaschii* transcripts (Wilting et al. 1997; Rother et al. 2001). In addition, inspection of the *M. jannaschii* genome revealed a putative archaeal SelB homolog, which was able to bind guanosine nucleotides and aminoacyl-$tRNA^{Sec}$ (Rother et al. 2000), suggesting that it is a key component of selenoprotein synthesis machinery in archaea.

6.3.4 Selenoproteins in Bacteria and Archaea

Most intracellular Se is found in selenoproteins in the form of Sec. Since selenol is highly nucleophilic and Sec is mostly deprotonated at physiological pH (pK_a: 5.2 for Sec vs. 8.3 for cysteine, Cys), Sec is more reactive than Cys (Zinoni et al. 1987; Axley et al. 1991). Therefore, due to the chemical properties of Se, almost all selenoproteins with Sec residues in their active site participate in intracellular redox systems (Table 6.1). The membrane-bound formate:hydrogen lyase-linked formate dehydrogenase H (FdhH encoded by *fdhF*) from *E. coli* was cloned, representing the first prokaryotic gene encoding a Sec residue, which is encoded by UGA (Zinoni et al. 1986). In *E. coli*, two other selenoprotein formate dehydrogenases, FdhO and FdhN, have been identified (Sawers et al. 2004). FDHs represent the most widespread selenoproteins in bacteria and archaea (Peng et al. 2016). FDH catalyzes the reversible oxidation of formate to CO_2 and is involved in energy metabolism, carbon

Table 6.1 Selenoproteins in bacteria

Selenoproteins	Gene	Characteristic organism	References
Formate dehydrogenase α subunit	*fdhA*	*E. coli, etc.*	Cox et al. (1981)
Selenophosphate synthetase	*selD*	*Eubacterium acidaminophilum, etc.*	Gursinsky et al. (2008)
Glycine reductase protein A	*grdA*	*Clostridium sticklandii, etc.*	Cone et al. (1976)
Glycine reductase protein B	*grdB*	*E. acidaminophilum, etc.*	Wagner et al. (1999)
Proline reductase	*pr*	*C. sticklandii, etc.*	Kabisch et al. (1999)
Sarcosine reductase	–	*E. acidaminophilum, etc.*	Hormann and Andreesen (1989)
Betaine reductase	–	*E. acidaminophilum, etc.*	Meyer et al. (1995)
Coenzyme F_{420}-reducing hydrogenase α subunit	*frhA*	*Syntrophobacter fumaroxidans, etc.*	Zhang et al. (2006)
Coenzyme F_{420}-reducing hydrogenase δ subunit	*frhD*	*S. fumaroxidans, etc.*	Zhang et al. (2006)
Heterodisulfide reductase subunit A	*hdrA*	*S. fumaroxidans, etc.*	Zhang et al. (2006)
Thioredoxin	*trx*	*Treponema denticola, etc.*	Kim et al. (2015)
Glutaredoxin	*grx*	*Clostridium* sp., *etc.*	Kim et al. (2011)
Peroxiredoxin	*prx*	*E. acidaminophilum, etc.*	Sohling et al. (2001)
Prx-like thiol:disulfide oxidoreductase	–	*Geobacter metallireducens, etc.*	Zhang et al. (2006)
Thiol:disulfide interchange protein	–	*Syntrophus aciditrophicus, etc.*	Zhang et al. (2006)
Fe-S oxidoreductase	*glpC*	*S. fumaroxidans, etc.*	Zhang et al. (2006)
NADH oxidase	–	*G. metallireducens*	Zhang et al. (2006)
Methionine sulfoxide reductase	*msrA*	*Clostridium* sp., *etc.*	Kim et al. (2009), etc.
Electron transfer protein	*prdC*	*C. sticklandii*	Fonknechten et al. (2010)
Glutathione peroxidase	*gpx*	*Treponema denticola*	Zhang et al. (2006)
HesB-like	–	*S. fumaroxidans, etc.*	Zhang et al. (2006)
SelW-like	–	*Desulfotalea psychrophila, etc.*	Zhang et al. (2006)
AhpD-like	–	*Alkaliphilus metalliredigenes*	Zhang et al. (2006)
ArsC-like	–	*D. psychrophila*	Zhang et al. (2006)
DsbA-like	–	*Anaeromyxobacter dehalogenans*	Zhang et al. (2006)
DsbG-like	–	*Symbiobacterium thermophilum*	Zhang et al. (2006)
DsrE-like	–	*Desulfovibrio vulgaris*	Zhang et al. (2006)
Homolog of AhpF	–	*Carboxydothermus hydrogenoformans*	Zhang et al. (2006)
Distant AhpD homolog	–	*Geobacter uraniumreducens*	Zhang et al. (2006)

fixation, and pH homeostasis (Ferry 1990). It contains an Fe-S cluster and either Mo or W (Andreesen and Makdessi 2008) coordinated by a pterin cofactor.

Besides *E. coli*, several selenoproteins have been characterized in *Eubacterium acidaminophilum* and *Clostridium sticklandii*, such as glycine reductase proteins A and B (Cone et al. 1976; Hormann and Andreesen 1989; Dietrichs et al. 1991; Garcia and Stadtman 1992; Meyer et al. 1995; Fonknechten et al. 2010). The glycine reductase system is essential for acetate formation via glycine; it comprises three proteins: glycine reductase proteins A, B, and C. The substrate-binding glycine reductase protein B is encoded by two genes, *grdB* and *grdE* (Wagner et al. 1999). Glycine reductase A (GrdA) is a small acidic, redox-active protein, which accepts the carboxymethyl group from GrdB. Another selenoprotein, D-proline reductase, appears to be similar to glycine reductase protein B and proline reductase B (PrdB) contains Sec in a motif similar to that found in GrdB (Kabisch et al. 1999).

In addition to experimentally verified selenoproteins, bioinformatics analyses have predicted the presence of additional selenoproteins from DNA sequence data (Zhang et al. 2006). A study shows that formate dehydrogenase α subunit (FdhA) and SelD are the most widespread selenoproteins in bacteria and that the bacterium *Syntrophobacter fumaroxidans* contains the largest number of selenoprotein genes among the bacteria that have been genome-sequenced. *S. fumaroxidans* has 31 selenoprotein-encoding genes including 1 for *selD*, 6 for *fdhA*, 3 for *frdA*, 8 for *frhD*, 7 for *hdrA*, 3 for *glpC*, *prx*, *hesB-like*, and *msrA*. Although those bioinformatics analyses provided a number of predicted selenoprotein genes, most gene products have not yet been experimentally characterized.

Several selenoproteins have been identified in methanogenic archaea (Table 6.2) (Jones et al. 1979; Yamazaki 1982; Halboth and Klein 1992; Vorholt et al. 1997; Wilting et al. 1997). The only archaea for which the presence of selenoproteins has been suggested, by either experimentation or prediction from genome sequence data, are methanogens dependent on the hydrogenotrophic methanogenesis pathway (Rother et al. 2001; Kryukov and Gladyshev 2004). However, not all hydrogenotrophic methanogens employ Sec. Within archaeal species, selenoproteins appear to be restricted to two genera, *Methanococcus* and *Methanopyrus*, according to an analysis of 56 available genome sequences representing 43 genera (Rother et al. 2001; Kryukov and Gladyshev 2004). Genome sequence analyses, radioactive *in vivo* labeling, and mutational studies identified at least six methanogenesis-related selenoproteins in *Methanococcus jannaschii*, *M. voltae*, *M. maripaludis, M. vannielii*, and *M. kandleri*. Selenophosphate synthetase (SelD) was identified as a selenoprotein, suggesting that selenoproteins synthesis is regulated by a selenoprotein itself (Wilting et al. 1997).

Table 6.2 Selenoproteins in archaea

Selenoproteins	Gene	Characteristic organism	References
Formate dehydrogenase	*fdhA*	*Methanococcus jannaschii*	Wilting et al. (1997)
		M. vannielii	Jones et al. (1979)
Selenophosphate synthetase	*selD*	*M. jannaschii*	Wilting et al. (1997)
Heterodisulfide reductase	*hdrA*	*M. jannaschii*	Wilting et al. (1997)
Formyl-methanofuran dehydrogenase	*fwuB*	*M. jannaschii*	Wilting et al. (1997)
		M. kandleri	Vorholt et al. (1997)
F_{420}-reducing hydrogenase	*fruA*	*M. jannaschii*	Wilting et al. (1997)
		M. voltae	Halboth and Klein (1992)
F_{420}-non-reducing hydrogenase	*vhuD/U*	*M. jannaschii*	Wilting et al. (1997)
		M. voltae	Halboth and Klein (1992)
		M. maripaludis	Sorgenfrei et al. (1993)
HesB-like protein	–	*M. jannaschii*	Kryukov and Gladyshev (2004)

6.3.5 Seleno-Amino Acids Metabolism

Three forms of seleno-amino acids have been identified in bacteria: Sec, selenocystine, and SeMet. The enzyme L-Sec lyase exists in various aerobic bacteria (Chocat et al. 1983) and was purified from *Citrobacter freundii* (Chocat et al. 1985). This enzyme exclusively catalyzes the pyridoxal 5′-phosphate (PLP)-dependent decomposition of L-Sec to L-alanine and elemental Se. On the other hand, *E. coli* possesses three cysteine desulfurases, IscS, SufS, and CsdB, which can utilize both L-Sec and L-Cys as a substrate (Mihara et al. 2000). Purified IscS from *E. coli* has been shown in an *in vitro* assay to supply Se from L-Sec for the synthesis of selenophosphate by SelD (Lacourciere et al. 2000). Another seleno-amino acid-acting enzyme, D-selenocystine α,β-lyase, which PLP-dependently decomposes D-selenocystine into pyruvate, ammonia, and elemental Se, was found in the anaerobic bacteria *C. sticklandii* and *C. sporogenes*. The enzyme was purified from *C. sticklandii* and characterized (Esaki et al. 1988). It consists of two subunits and has a molecular mass of approximately 74 kDa. In addition to D-selenocystine, other analogous amino acids can be used as substrates including D-cystine, D-lanthionine, *meso*-lanthionine, and D-cysteine. The biological roles of both L-Sec lyase and D-selenocystine α,β-lyase remain unknown. As Se has a very limited availability as a trace element for microorganisms, these enzymes are proposed to aid in recycling Se from degraded selenoproteins containing L-Sec to make new selenoproteins (Tamura et al. 2004).

L-Sec is the predominant seleno-amino acid in bacteria, but SeMet is also incorporated into proteins (Berntsson et al. 2009). Unlike L-Sec, SeMet is randomly

incorporated into proteins instead of methionine because there is no specific machinery for selenomethionine. The incorporation of SeMet in place of Met is not expected to affect protein function. L-Methionine γ-lyase, which has been purified from *Pseudomonas putida*, decomposes SeMet into α-ketobutyrate, ammonia, and methaneselenol (Esaki et al. 1979). Recently, SeMet has been used in probiotic supplements (Krittaphol et al. 2011; Gojkovic et al. 2014) and in experimental analyses such as X-ray crystallography (Berntsson et al. 2009).

6.3.6 Other Selenium Compounds in Bacteria

Se is used for incorporation into selenoproteins as Sec and is also found in several bacterial tRNAs. The modified tRNA nucleoside, 5-methylaminomethyl-2-SeU, is located at the wobble position in the anticodons of $tRNA^{Lys}$, $tRNA^{Glu}$, $tRNA^{Pro}$, and $tRNA^{Gln}$. The modification likely contributes to tRNA recognition and translation efficiency (Chen and Stadtman 1980; Wittwer et al. 1984; Ching et al. 1985a, b; Wolfe et al. 2004). SeU is generated by the specific substitution of Se for sulfur in 2-thiouridine by tRNA 2-SeU synthase (YbbB) (Veres and Stadtman 1994), where selenophosphate serves as a Se donor (Veres et al. 1992; Glass et al. 1993). Mutants of *E. coli* and *S. typhimurium* containing a defective *selD* gene are unable to incorporate Se into proteins and tRNAs (Kramer and Ames 1988; Stadtman et al. 1989).

Comparative genomic and phylogenetic analyses showed the possibility that Se is used in Se-dependent molybdenum hydroxylases (SDMH) as a third pathway of Se utilization in bacteria and archaea (Haft and Self 2008; Zhang et al. 2008). In this pathway, the SelD protein may activate Se for SDMH maturation via two proteins, YqeB and YqeC, whose functions are still unknown. Three SDMHs have been characterized from two species: nicotinic acid hydroxylase and xanthine dehydrogenase from *Eubacterium barkeri* (Gladyshev et al. 1994; Schräder et al. 1999) and xanthine dehydrogenase and purine hydroxylase from *Clostridium purinolyticum* (Self and Stadtman 2000; Self et al. 2003).

Besides the above seleno-molecules, several minor Se-containing molecules have been identified in bacteria, such as Se exopolysaccharide (Ding et al. 2014) and Se-containing phycocyanin (Se-PC) (Chen and Wong 2008). Se-PC was identified and purified from Se-enriched *Spirulina platensis* (Chen and Wong 2008). Se-PC has stronger antioxidant activity than phycocyanin and it shows dose-dependent protective effects against H_2O_2-induced oxidative DNA damage in erythrocytes. Although it is artificial, Se-containing exopolysaccharides have been obtained using a *Rhizobium* sp. N6113 exopolysaccharide (Ding et al. 2014). These novel organic Se species have been proposed for use in antitumor chemoprevention applications.

6.3.7 Selenium Detoxification

As mentioned above, Se oxyanions exhibit toxicity to living organisms. Some bacteria relieve Se toxicity by glutathionylation and methylation of Se compounds. Glutathione is the most abundant low molecular weight thiol in the cell and the reduction of selenite with GSH, producing GS-Se-SG and glutathioselenol, was demonstrated (Ganther 1968; Ganther 1971). In addition to glutathionylation, methyl-selenides and methyl-selenoxides are common degradation and detoxification products for toxic Se oxyanions. The volatile forms, dimethyl selenide (DMSe) and dimethyl diselenide (DMDSe), are 500–700 times less toxic than other Se derivatives (Ganther et al. 1966). The conversion of inorganic and organic Se compounds to their volatile forms by microorganisms was first observed using lake water and sediment (Chau et al. 1976). *Rhodocyclus tenuis* and *Rhodospirillum rubrum* can produce both DMSe and DMDSe from selenate while growing phototrophically, and *R. tenuis* also produces DMSe from selenite (McCarty et al. 1993). One is the bacterial thiopurine methyltransferase (bTPMT) encoded by the *tpm* gene of *Pseudomonas syringae* (Ranjard et al. 2002). The enzyme is involved in the conversion of selenite and Se-methyl-Sec into DMSe and DMDSe. The other is a calicheamicin methyltransferase homolog encoded by the *mmtA* gene (Ranjard et al. 2004). Free SeMet is also converted to DMSe and DMDSe via the pathway including bTPMT (Ranjard et al. 2003). In addition, a recent report shows that *P. stutzeri* NT-I aerobically transform selenate, selenite, and biogenic elemental Se into DMSe and DMDSe; these volatile forms were temporarily accumulated in the aqueous phase and then transferred into the gaseous phase (Kagami et al. 2013). Demethylation of DMSe in anaerobic Se-contaminated sediments was reported to proceed via methanogenic pathways established for growth on dimethylsulfide (Oremland and Zehr 1986).

6.3.8 Transport of Selenium Compounds

Until now, two types of sulfate transporters have been demonstrated to transport Se oxyanions: the sulfate-thiosulfate permease type (Turner et al. 1998) and the SulP-type permease type (Zolotarev et al. 2008). Sulfate-thiosulfate permeases belong to the sulfate/tungstate uptake transporter (SulT) family of the ABC transporter superfamily. In *E. coli* and *Salmonella typhimurium*, two types of SulT sulfate-thiosulfate permeases were identified. They consist of: (1) periplasmic proteins Sbp, (sulfate-binding protein, Pflugrath and Quiocho 1985) and CysP (thiosulfate-binding protein, Hryniewicz et al. 1990); (2) membrane proteins CysT and CysW (Sirko et al. 1990); (3) ATP-binding protein CysA (Sirko et al. 1990). The SulP sulfate permease superfamily is a large and ubiquitous protein family with hundreds of sequenced members derived from all three domains of life. However, only a few proteins in this family have been functionally characterized (Kertesz 2001; Saier et al. 2006).

Selenate transport through the SulP sulfate permease has also been reported in *Mycobacterium tuberculosis* (Zolotarev et al. 2008) and in *Cupriavidus metallidurans* CH34 (Avoscan et al. 2009).

Recent genomic analyses suggest that the membrane protein YedE may also be involved in Se transport (Lin et al. 2015). This protein, exclusively found in Se-utilizing organisms, contains ten transmembrane domains and shows distant similarity to a sulfur transporter (Gristwood et al. 2011). The sulfur-related transporters contain several conserved glycines and an invariant Cys, which is probably an important functional residue. The *yedE* gene locus is often located next to known Se-related genes such as SirA-like, SelD, and Sec lyase in many bacteria of different phyla, implying that the protein may be involved in Se metabolism. A similar relationship between YedE and SelD was observed in archaea, and in this domain, the *yedE* gene is split into two adjacent genes.

6.4 Biotechnological Applications of Bacterial Selenium Metabolism

6.4.1 Production of Functional Materials by Microbial Biofactories

Microbial metabolism can be harnessed for the production of functional materials that find multiple industrial and domestic applications. Se^0 and metal selenide particles possess photo-optical and semiconducting physical properties required in devices such as photocopiers and microelectronic circuits (Oremland et al. 2004). The bulk of the Se^0 particles formed during dissimilatory reduction of Se oxyanions are not randomly shaped blobs of amorphous material, but instead are discretely shaped spheres of nano-sized dimensions (~200–400 nm). The spheres first accumulate on cell surfaces and then are sloughed off into the surrounding medium (Fig. 6.5) (Oremland et al. 2004). Washed nano-spheres recovered from 3 physiologically and

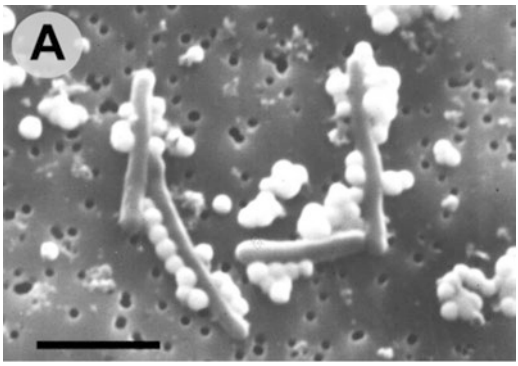

Fig. 6.5 Formation of Se^0 nanospheres on the surface of *B. selenitireducens* that slough off into the extracellular medium. Scale bar = 1 micrometer. (Adapted from Oremland et al. (2004) with permission)

phylogenetically different Se-respirers (*S. barnesii, B. selenitireducens*, and *Selenihalanaerobacter shriftii*) exhibited different spectral properties, which in turn were different from chemically precipitated Se^0. It was also found that washed Se-nanospheres were unable to undergo further reduction to HSe^-, whereas freshly-formed material could. Subsequent studies found that the nano-spheres were enveloped by a diaphanous layer, some of which consisted of peptides that are essential for bacterial adhesion to the materials and ultimately for their spherical shapes and sizes (Lenz et al. 2011; Debieux et al. 2011; Jain et al. 2015; Staicu et al. 2015c; Gonzalez-Gil et al. 2016). Over the past 12 years, considerable scientific interest has been piqued by the phenomenon of bacterial Se^0 nanospheres and many different species of prokaryotes (and microscopic eukaryotes like yeasts) have been found to be able to generate them, as summarized by Shirsat et al. (2015). Most of the described organisms carry out a reductive detoxification reaction when exposed to Se(IV) and do not use Se oxyanions as terminal electron acceptors.

One particular microorganism, *Veillonella atypica*, can form HSe^- by reduction of either Se(+IV) or Se(VI) oxyanions (Pearce et al. 2008, 2011). This organism is particularly attractive because reduction does not proceed through formation of Se^0 and thus the formed HSe^- anions can be precipitated with countering divalent cations of interest (e.g. Zn and Cd) and harvested as truly nanosized particles (~3–5 nm range). It remains to be determined whether or not they can somehow be placed uniformly in nano-arrays ("quantum-dots") that allows for quantum photonic effects to take place and make them suitable for practical applications in nano-technology (Fellowes et al. 2013; Mal et al. 2016). Nonetheless, use of microorganisms rather than harsh chemicals to form nanomaterials of Se and other Group 16 (G16) elements such as tellurium (Baesman et al. 2007, 2009) holds promise of a "green" technology that can produce nano-sized materials, perhaps with unique properties (Nancharaiah and Lens 2015).

6.4.2 *Biofortification of Plant Species Using Selenium-Reducing Bacteria*

Plant biofortification is a strategy which aims to increase the nutritional value and micronutrient levels (e.g. Se) in the edible parts of crop species (Wu et al. 2015). For this, various approaches can be used including conventional selective breeding, genetic engineering, Se fertilizers or microbial-mediated soil inoculation. Crops are the major source of dietary Se worldwide and may be employed to extract this element from seleniferous soils, thus providing dietary Se in low-Se areas. Se accumulator plant species have been shown to accumulate 100–1000 mg/kg dry weight (DW) Se and Se hyperaccumulators can even accumulate 1000–15,000 mg/kg DW Se on seleniferous soils, that is 0.1–1.5% (El Mehdawi and Pilon-Smits 2012). Endophytic bacteria were shown to have plant growth promoting properties and

displayed extremely high tolerance to toxic Se oxyanions by forming red Se^0 particles (Fig. 6.6) (Sura-de Jong et al. 2015). In numerous studies, beneficial bacteria have been inoculated to plant growth medium in an attempt to increase the nutritional value and stimulate biomass production of the plants. Se accumulator *Brassica juncea* (Indian mustard) was inoculated with two Se-tolerant bacterial consortia (G1 and G2) and was shown to accumulate Se to 711 mg/kg DW in leaves, 276 mg/kg DW in pod husks, and 358 mg/kg DW in seeds (Yasin et al. 2015a). Plants inoculated with bacterial consortium G1 showed significantly increased growth (dry biomass and seed weight) as compared to control plants and G2-inoculated plants (Yasin et al. 2015a). Furthermore, the growth of *B. juncea* was stimulated by 1.7-fold using a novel Se-tolerant bacterium, *P. moraviensis* stanleyae, although no significant effect on Se accumulation was observed (Staicu et al. 2015b). In another study, the inoculation of wheat (*Triticum aestivum* L.) with YAM2 cultures, a bacterium with 99% similarity to *Bacillus pichinotyi*, resulted in enhanced plant growth. YAM2-inoculated wheat plants showed significantly higher dry weight, shoot length, and spike length compared to un-inoculated plants and significantly higher Se concentration in wheat kernels (167%) and stems (252%) (Yasin et al. 2015b). The significance of these studies stems mainly from the fact that wheat is a staple food for humans and animals and therefore a useful Se-delivery vehicle. In another study, Se biofortification of wheat plants using endophytic bacteria was shown to lead not only to enhanced plant growth, but could also act as a control strategy against pests such as *Gaeumannomyces graminis*, the principal soil-borne fungal pathogen in volcanic soils from southern Chile (Durán et al. 2014). Plant biofortification using Se-reducing bacterial inocula is an emerging research topic in need of further research and clarification. The exact contribution of bacteria to plant growth enhancement and micronutrient accumulation are open questions to be answered by future studies. In addition, it will be valuable to investigate to what extent Se-bacteria are colonizing plant organs and whether this colonization is an important factor for plant Se accumulation and speciation.

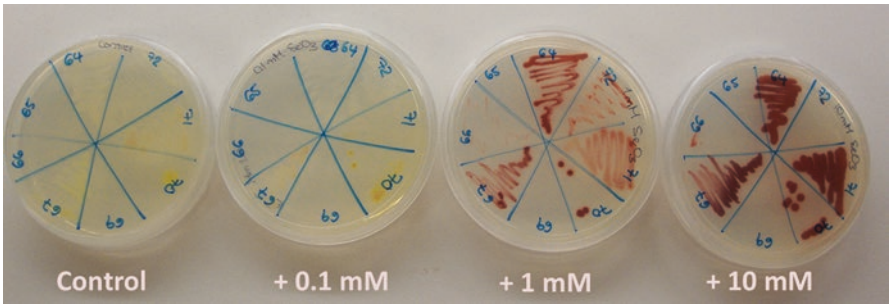

Fig. 6.6 Red Se^0 produced by endophytic bacteria isolated from the root tissue of *Stanleya pinnata* and *Astragalus bisulcatus* that were exposed to progressively higher concentrations [0.1, 1, and 10 mM] of SeO_3^{2-} (as Na_2SeO_3). Control represents a plate containing Luria Bertani growth medium without selenite

6.5 Conclusions

The relationship between bacteria and Se predates the Oxygen Revolution (~2.3 Ga). A large number of sequenced bacterial genomes indicate Se is an ancient trait once common for all bacterial species. Depending on several factors such as bacterial species, enzymatic repertoire, and geochemical context (e.g., oxygen profile, redox conditions, and nutrient availability) bacteria can exploit Se to energize its metabolic machinery, but it can also be affected by the toxicity exhibited by various forms of Se. On the other hand, being an essential micronutrient, a complex molecular mechanism is used by bacteria and archaea to incorporate Se into various cellular components (e.g. amino acids and proteins). Specifically, Se is involved in three major metabolic strategies employed by bacteria: *assimilatory* metabolism (biosynthesis), *dissimilatory* metabolism (energy generation), and detoxification. Several biotechnological applications using Se microbial specialists show high potential for the biofabrication of functional materials (e.g. Se^0 nanoparticles and quantum dots) and for the biofortification of crop species.

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