

# Chapter 3



## Microbial reduction of selenium oxyanions: energy-yielding and detoxification reactions

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### 3.1 INTRODUCTION

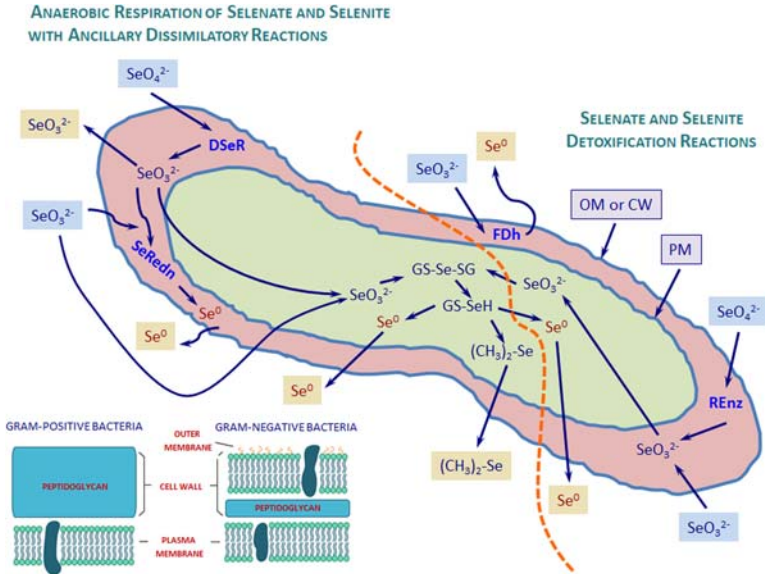
Selenium (Se), a semi-metallic chemical element in the oxygen group (group 16 [VIa]) of the periodic table, can be beneficial – even essential in some instances – for microbes and animals, including humans, when present at a suitable concentration, whereas no essential Se requirement has been shown for higher plants (Lenz & Lens, 2009; Winkel *et al.*, 2015). Se is an essential trace element required for the biosynthesis of seleno-amino acids such as selenocysteine (Se-Cys) (Bock *et al.*, 1991; Gromer *et al.*, 2005) and selenomethionine (Se-Met, the major dietary form) (Schrauzer, 2000). These are potent antioxidants as well as a source of Se for the synthesis of Se-dependent antioxidant and repair proteins such as glutathione peroxidases, thioredoxin reductases, and methionine sulfoxide reductases (Flohe *et al.*, 1973; Kim & Gladyshev, 2007; Mustacich & Powis, 2000; Zoidis *et al.*, 2018). Multiple selenoproteins have been identified in eukaryotes, ranging from yeasts (Tastet *et al.*, 2008) to humans (Papp *et al.*, 2018), but Se is also found in prokaryotic proteins such as formate dehydrogenase from *Methanococcus jannaschii* (Jones *et al.*, 1983) formylmethanofuran dehydrogenase from *Methanopyrus kandleri* (Vorholt *et al.*, 1997), and thiol/disulfide oxidoreductase from *Geobacter sulfurreducens* (Kryukov & Gladyshev, 2004).

Although Se is an essential mineral element, there is a very narrow window between necessary and toxic concentrations. The oxyanions selenite ( $\text{SeO}_3^{2-}$ ) and selenate ( $\text{SeO}_4^{2-}$ ) are the primary Se species found in oxic environments. They are highly soluble and bioavailable, which makes them toxic at low concentrations in the parts per million range (Lenz & Lens, 2009; Nancharaiah & Lens, 2015). Selenite is the more toxic of the two species (Frankenberger & Engberg, 1998). This makes it imperative to understand how the distribution of Se in the environment is controlled. Such an understanding will lead to efficient strategies for the detoxification of environmental matrices contaminated with Se, including soil, sediment, surface water, groundwater and wastewater.

Selenium behaves chemically in a similar manner to sulfur (S). Both Se and S can exist in the  $2^-$ , 0,  $4^+$  and  $6^+$  oxidation states and can therefore form structurally analogous compounds, although those containing Se are more toxic because Se has a lower electronegativity than S and forms weaker bonds (Whitham, 1995). The concentration, speciation and association of Se in a given habitat depends on the pH and redox conditions, the solubility of Se salts, the complexing ability of soluble and solid ligands, biological interactions, and reaction kinetics. Redox transformations that occur in natural systems can increase or decrease the mobility and bioavailability of Se, and can involve both chemical and biotic mechanisms (Myneni *et al.*, 1997; Zhang *et al.*, 2004b).

Prokaryotic and eukaryotic microbes play a prominent role in the biogeochemical cycle of Se by performing both oxidation and reduction reactions (Nancharaiah & Lens, 2015; Ojeda *et al.*, 2020). Se metabolic transformations have been reported in all domains of life – *Bacteria* (Böck, 2001), *Archaea* (Bini, 2010), and *Eukarya* (Rayman, 2012) – as well as in viruses (Shisler *et al.*, 1998). Microbes can transform Se oxyanions via assimilatory or dissimilatory reduction mechanisms as well as alkylation/dealkylation and oxidation reactions (Ojeda *et al.*, 2020). The main bacterial mechanisms of selenate and selenite reduction are shown in Figure 3.1.

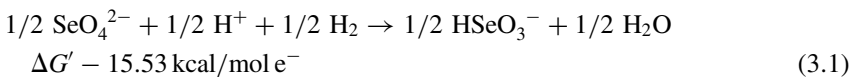
Assimilatory reduction refers to the incorporation of Se into seleno-amino acids, whereas dissimilatory reduction primarily involves the anaerobic respiration of selenite or selenate for energy production. If the reduction of Se oxyanions occurs in the absence of energy generation, it is interpreted as a detoxification mechanism. Prokaryotes can use selenite and selenate as terminal electron acceptors, resulting in the formation of insoluble, nanostructured elemental  $\text{Se}^0$  (Stolz & Oremland, 1999). Some bacteria and fungi can also reduce Se oxyanions to  $\text{Se}^0$  under aerobic or microaerophilic conditions as a detoxification reaction (Tejo Prakash *et al.*, 2009) or, in phototrophic bacteria, as a redox homeostasis mechanism (Kessi *et al.*, 1999).

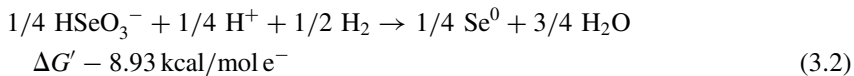


**Figure 3.1** The dissimilatory reduction of selenate and selenite by anaerobic respiration (or other energy uncoupled reactions) and selenium detoxification mechanisms in bacteria. Abbreviations: CW cell wall;  $(\text{CH}_3)_2\text{-Se}$  dimethylselenide; DSeR dissimilatory selenate reductase; FDh fumarate reductase; GS-Se-SG selenodiglutathione; GS-Se-H selenoglutathione; OM outer membrane; PM plasma membrane; REnz reductase enzyme; seRedn selenite reductase.

### 3.2 SELENIUM OXYANIONS AS FINAL ELECTRON ACCEPTORS IN BACTERIAL ENERGY METABOLISM

Bacterial strains that support their growth by using selenate and/or selenite for respiration under anaerobic/anoxic conditions are known as selenium-respiring bacteria (Nancharaiyah & Lens, 2015). The reduction potentials, under standard conditions, for the couples  $\text{SeO}_4^{2-}/\text{SeO}_3^{2-}$  and  $\text{SeO}_3^{2-}/\text{Se}^0$  are +0.48 and +0.21 V, respectively. This is lower than the potential required for nitrate reduction ( $\text{NO}_3^-/\text{N}_2$ ) but much higher than that required for sulfate reduction  $\text{SO}_4^{2-}/\text{SO}_3^{2-}$ . The free energies for the reduction of selenate and selenite coupled to  $\text{H}_2$  oxidation are (Newman *et al.*, 1998; Stolz & Oremland, 1999):





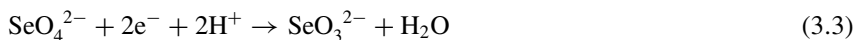
The reduction of selenate, therefore, occurs at a slightly lower redox potential than that required for nitrate reduction but at a higher redox potential than sulfate reduction. Thermodynamic values show that selenate reduction is energetically favorable for microorganisms and can thus provide a significant mechanism for certain bacteria to conserve energy in natural environments.

The dissimilative reduction of selenate to  $\text{Se}^0$  was first reported in sediment slurry experiments (Oremland *et al.*, 1989). The complete reduction of millimolar concentrations of selenate into quantitative levels of  $\text{Se}^0$  was observed under anaerobic conditions. It was accelerated in the presence of  $\text{H}_2$  or acetate but inhibited by autoclaving or in the presence of  $\text{O}_2$ ,  $\text{NO}_3^-$  or  $\text{MnO}_2$ , but not by sulfate. The dissimilative reduction of selenate was clearly shown to occur independently of sulfate, indicating that the Se and S biogeochemical cycles are not related. Subsequently, enriched cultures from agricultural drainage waters in the San Joaquin Valley (California, USA) yielded two bacterial strains that metabolized Se in axenic cultures (Macy *et al.*, 1989). One was a strain named *Pseudomonas* sp. AX that reduced selenate to selenite by respiration, and the other was a strictly anaerobic, Gram-positive, rod-shaped bacterium (strain E) that reduced selenite to  $\text{Se}^0$  under anaerobic growth conditions.

Further studies have identified other prokaryotes that can use selenate and/or selenite as final electron acceptors for dissimilative reduction, including phylogenetically diverse groups of Gram-positive and Gram-negative *bacteria* as well as *archaea* (Oremland & Stolz, 2000; Stolz *et al.*, 2002). This indicates that Se respiration is a widespread phenomenon.

### 3.2.1 Bacterial selenate respiration

Bacterial selenate respiration requires the sequential reduction of the Se oxyanions selenate and selenite, resulting in the precipitation of red-colored  $\text{Se}^0$  and the formation of Se nanostructures. The overall process for the biological reduction of selenate to  $\text{Se}^0$  is described by two sequential reactions (Debieux *et al.*, 2011; Nancharaiah & Lens, 2015):



The respiration of Se oxyanions to  $\text{Se}^0$  is followed by the formation of Se nanostructures. Selenate respiration is driven by metalloproteins that utilize cofactors containing iron and molybdenum, and this has been well described in a few model bacterial strains (Butler, 2012). Analysis of the processes concerning the formation of Se nanostructures is widely discussed elsewhere (see Chapter 10).

Multiple bacterial strains are capable of respiring selenate under anaerobic conditions. These have mainly been sourced from aquifers with high levels of Se pollution and were isolated in selenate respiration enrichment cultures. They mainly represent the phyla *Proteobacteria*, *Firmicutes* and *Chrysiogenetes* (Table 3.1). Different organic substrates can serve as electron donors in the respiratory conversion of selenate to  $\text{Se}^0$  (Narasingarao & Häggblom, 2007a; Stolz & Oremland, 1999). Both  $\text{H}_2$  and methane can drive the reduction of selenate under anaerobic conditions (Haroon *et al.*, 2013; Orphan *et al.*, 2001; Shi *et al.*, 2020; Stolz & Oremland, 1999). *Gammaproteobacteria* isolated via enrichment cultures from sediments of Arthur Kill and the Kesterson Reservoir (San Joaquin Valley, California, USA) were also found to be capable of selenate respiration while using aromatic compounds such as benzoate, 3-hydroxybenzoate or 4-hydroxybenzoate as a carbon source (Knight *et al.*, 2002).

*Thauera selenatis* AX<sup>T</sup> was isolated from the Se-contaminated drainage waters of the San Joaquin Valley and was initially classified as the first member of a new species of  $\beta$ -*Proteobacteria*: *T. selenatis* sp. (Macy *et al.*, 1993). *T. selenatis* AX<sup>T</sup> was found to grow under anaerobic conditions using selenate as the sole electron acceptor. Moreover, it could simultaneously reduce both selenate and nitrate, thus indicating that distinct reductases were acting on each substrate (Rech & Macy, 1992). However, it was unable to use selenite as a final electron acceptor. The *T. selenatis* AX<sup>T</sup> selenate reductase (Ser) was purified and characterized. It is a soluble metalloenzyme, located in the periplasmic space, that possesses both iron and molybdenum redox centers (Shröder *et al.*, 1997). Ser is a trimeric complex of the proteins SerA, SerB and SerC. A fourth component (SerD) is a cytoplasmic chaperone probably involved in the insertion of the molybdenum cofactor into the catalytic subunit SerA. SerB is an iron-sulfur protein which transfers electrons from SerC to SerA, whereas SerC is a cytochrome *b*. Electron paramagnetic resonance (EPR) spectroscopy revealed that SerA and SerB contain cysteine-rich motifs (Dridge *et al.*, 2007). The cysteine-rich motif in SerA is proposed to bind a [4Fe–4S] iron–sulfur cluster, whereas the multiple cysteine-rich motifs in SerB are predicted to bind one [3Fe–4S] and three [4Fe–4S] iron–sulfur clusters. The reduction of selenate to selenite requires the transfer of two electrons from quinole ( $\text{QH}_2$ ) and the presence of a cytochrome *c4* protein (Lowe *et al.*, 2010). The mechanism of electron transfer to periplasmic cytochrome *c4* is proposed to involve both quinol cytochrome *c* oxidoreductase (QCR) and quinol dehydrogenase (QDH). A periplasmic nitrite reductase may be involved in the reduction of selenite into  $\text{Se}^0$  under anaerobic conditions (DeMoll-Decker & Macy, 1993). However, once selenite has passed the cytoplasmic membrane it can be reduced to  $\text{Se}^0$  via a thiol-mediated mechanism that probably involves glutathione (GSH) (Debieux *et al.*, 2011). The protein SefA, isolated from the spent medium of *T. selenatis* AX<sup>T</sup> grown under aerobic conditions, was shown to be involved in the stabilization of the  $\text{Se}^0$

Table 3.1 Bacterial strains capable of selenate and/or selenite respiration to sustain cell growth (n.d. not determined).

Microbial Strain	Taxonomical Classification	Source	Electron Donors	Electron Acceptors	Formation/Location of Se Nanostructures	Reference
<i>Thauera selenatis</i> AX <sup>T</sup> (ATCC 55363 <sup>T</sup> )	$\beta$ -Proteobacteria	Drainage waters of the San Joaquin Valley, CA, USA	Acetate, H <sub>2</sub> , O <sub>2</sub>	SeO <sub>4</sub> <sup>2-</sup> ; NO <sub>3</sub> <sup>-</sup>	Yes intracellular and extracellular	Debieux <i>et al.</i> , 2011; Macy <i>et al.</i> , 1993
<i>Enterobacter cloacae</i> SLD1a-1 (ATCC 700258)	$\gamma$ -Proteobacteria	Freshwater samples from the San Luis Drain, CA, USA	Glucose, Complex medium (TSB)	SeO <sub>4</sub> <sup>2-</sup> ; NO <sub>3</sub> <sup>-</sup> , O <sub>2</sub>	Yes extracellular	Losi and Frankenberger, 1997; Ridley <i>et al.</i> , 2006; Watts <i>et al.</i> , 2003
<i>Citrobacter freundii</i> RLS1	$\gamma$ -Proteobacteria	Se-contaminated sediment	Glucose, Acetate, Lactate	SeO <sub>4</sub> <sup>2-</sup> ; NO <sub>3</sub> <sup>-</sup> , O <sub>2</sub>	Yes	Zhang <i>et al.</i> , 2004a
<i>Feromonas putsuensis</i> FUT3661 <sup>T</sup>	$\gamma$ -Proteobacteria	Sediment from Tokyo Bay	Lactate, Pyruvate, Tryptone	SeO <sub>4</sub> <sup>2-</sup> ; S <sub>2</sub> O <sub>3</sub> <sup>3-</sup> , Fe(III), AsO <sub>4</sub> <sup>2-</sup> , MnO <sub>2</sub> ; S <sup>2-</sup> ; O <sub>2</sub>	Yes	Nakagawa <i>et al.</i> , 2006
<i>Feromonas kyonanensis</i> Asr22-7 <sup>T</sup>	$\gamma$ -Proteobacteria	Alimentary tract of littleneck clams from Tokyo Bay	Lactate, Pyruvate, Tryptone	SeO <sub>4</sub> <sup>2-</sup> ; Fe(III), AsO <sub>4</sub> <sup>2-</sup> , MnO <sub>2</sub> ; S <sup>2-</sup> ; O <sub>2</sub>	Yes	Nakagawa <i>et al.</i> , 2006
<i>Sedimenticola selenatireducens</i> AK4OH1	$\gamma$ -Proteobacteria	Estuarine sediments	Lactate, Pyruvate, Tryptone	SeO <sub>4</sub> <sup>2-</sup> ; Fe(III), AsO <sub>4</sub> <sup>2-</sup> , MnO <sub>2</sub> ; S <sup>2-</sup> ; O <sub>2</sub>	Yes	Narasingarao and Häggblom, 2006
<i>Sulfurospirillum bamesii</i> SES-3	$\delta$ -Proteobacterium	Se-contaminated marsh freshwater	Lactate, Pyruvate, Formate, Fumarate	SeO <sub>4</sub> <sup>2-</sup> ; NO <sub>3</sub> <sup>-</sup> , SO <sub>4</sub> <sup>2-</sup> ; Fe(III), AsO <sub>4</sub> <sup>2-</sup> ; O <sub>2</sub>	Yes	Oremland <i>et al.</i> , 1994; Stolz <i>et al.</i> , 1999

<i>Pelobacter seleniigenes</i> KM <sup>T</sup>	<i>δ-Proteobacterium</i>	Sediment from a wetland system	Acetate, Lactate, Pyruvate	SeO <sub>4</sub> <sup>2-</sup> ; NO <sub>3</sub> <sup>-</sup> Fe(III) S <sub>2</sub> <sup>-</sup>	Yes extracellular and cell-associated	Narasingarao and Häggblom, 2007b
<i>Bacillus arsenicoselenatis</i> E1H	<i>Firmicutes, Bacilli</i>	Anoxic muds of Mono Lake, CA, USA	Lactate	SeO <sub>4</sub> <sup>2-</sup> ; AsO <sub>4</sub> <sup>2-</sup>	Yes	Switzer-Blum <i>et al.</i> , 1998
<i>Bacillus selenatarsenatis</i> SF-1	<i>Firmicutes, Bacilli</i>	Wastewater sediment from a glass manufacturing plant	Lactate	SeO <sub>4</sub> <sup>2-</sup> ; NO <sub>3</sub> <sup>-</sup> AsO <sub>4</sub> <sup>2-</sup>	Yes intracellular and extracellular	Fujita <i>et al.</i> , 1997; Kuroda <i>et al.</i> , 2011
<i>Bacillus selenitireducens</i> MLS10	<i>Firmicutes, Bacilli</i>	Mono Lake, CA, USA	Lactate; Pyruvate	SeO <sub>3</sub> <sup>2-</sup> ; NO <sub>3</sub> <sup>-</sup> ; NO <sub>2</sub> <sup>-</sup> AsO <sub>4</sub> <sup>2-</sup> ; fumarate	Yes periplasmic and extracellular	Switzer-Blum <i>et al.</i> , 1998; Wells <i>et al.</i> , 2019
<i>Bacillus beveridgei</i> MLTeJB	<i>Firmicutes, Bacilli</i>	Sediment from Mono Lake, CA, USA	Lactate	SeO <sub>4</sub> <sup>2-</sup> ; SeO <sub>3</sub> <sup>2-</sup> ; NO <sub>3</sub> <sup>-</sup> ; NO <sub>2</sub> <sup>-</sup> AsO <sub>4</sub> <sup>2-</sup> ; TeO <sub>4</sub> <sup>2-</sup> ; TeO <sub>3</sub> <sup>-</sup>	Yes	Baesman <i>et al.</i> , 2009
<i>Desulfuribacillus sibiarsenatis</i> MLFW-2 <sup>T</sup>	<i>Firmicutes, Bacilli</i>	Sediment from the drainage area of a geothermal spring near Mono Lake, CA, USA	Lactate, Pyruvate, Formate, H <sub>2</sub>	SeO <sub>4</sub> <sup>2-</sup> ; SeO <sub>3</sub> <sup>2-</sup> ; NO <sub>3</sub> <sup>-</sup> ; NO <sub>2</sub> <sup>-</sup> DMSO	n.d.	Abin and Hollibaugh, 2017
<i>Selenihalanaerobacter shirfii</i> DSSe-1 ATCC BAA-73	<i>Firmicutes, Clostridia</i>	Dead Sea	Glucose, Glycerol	SeO <sub>4</sub> <sup>2-</sup> ; NO <sub>3</sub> <sup>2-</sup> ; NO <sub>2</sub> <sup>-</sup> AsO <sub>4</sub> <sup>2-</sup> ; S <sub>2</sub> <sup>2-</sup> ; fumarate	Yes	Blum <i>et al.</i> , 2001
<i>Desulfurispirillum indicum</i> S5	<i>Chrysiogenetes</i>	Sediment from an estuarine canal	Acetate, Lactate, Pyruvate	SeO <sub>4</sub> <sup>2-</sup> ; SeO <sub>3</sub> <sup>2-</sup> NO <sub>3</sub> <sup>-</sup> ; AsO <sub>4</sub> <sup>2-</sup>	Yes intracellular	Rauschenbach <i>et al.</i> , 2011

nanostructures by preventing aggregation and may also help in the secretion of these structures from the cell (Butler, 2012; Debieux *et al.*, 2011).

*Enterobacter cloacae* SLD1a-1 was isolated from freshwater samples from the San Luis Drain (California, USA). It is a facultative anaerobe that can use nitrate and selenate as terminal electron acceptors during anaerobic growth (Losi & Frankenberger, 1997). Selenate reduction is catalyzed by a membrane-associated reductase (Ser), and is followed by rapid expulsion of the Se<sup>0</sup> particles. Moreover, the strain was shown to reduce selenate and selenite under either anaerobic or aerobic conditions with no induction or conditioning process necessary, and the concomitant reduction of nitrate, selenate and selenite was also confirmed. The membrane-associated Ser protein is a molybdenum-dependent selenate reductase expressed under both aerobic and anaerobic conditions, with the catalytic site facing the periplasmic compartment (Watts *et al.*, 2003). The protein is selective for selenate and cannot reduce nitrate. Unlike *T. selenatis* AX<sup>T</sup> Ser, *E. cloacae* SLD1a-1 Ser is unable to support bacterial growth, and may instead be used for detoxification, in accordance with the location of the active site, enabling the bacterium to protect itself against elevated levels of both selenate and the reduction product selenite (Watts *et al.*, 2003). Isolation and characterization of the enzyme revealed that it is a heterotrimeric complex ( $\alpha\beta\gamma$ ) with an apparent molecular mass of ~600 kDa containing molybdenum, heme, and non-heme iron as prosthetic constituents and cytochrome *b* in the active complex (Ridley *et al.*, 2006).

*Citrobacter freundii* RLS1 (class  $\gamma$ -Proteobacteria, family Enterobacteriaceae) is similarly proposed to achieve the dissimilative reduction of selenate to Se<sup>0</sup> via the formation of selenite. This strain, isolated from a Se-contaminated sediment from the New River (California, USA), can grow on selenate as a final electron acceptor under microaerophilic conditions and starts to reduce selenate once O<sub>2</sub> is depleted in the medium, resulting in the formation of amorphous red selenium (Zhang *et al.*, 2004a, b). Genome sequencing and functional annotation suggested that selenate reduction is mediated by a membrane-bound metalloenzyme with a molybdenum cofactor, regulated by the FNR protein (fumarate nitrate reduction) that monitors the availability of oxygen in the cytoplasm. FNR also regulates selenate reductase in *E. cloacae* SLD1a-1 (Yee *et al.*, 2007). Genes for selenate reductase in the *C. freundii* genome are organized in the *ynfEGHdmsD* operon, which is induced by the FNR protein under anaerobic conditions. Once bound to the molybdenum cofactor, the selenate reductase protein folds into the appropriate conformation and anchors onto the inner membrane with the active site of the YnfE subunit facing the periplasmic compartment. Electrons are transferred from the menaquinone pool to YnfE via the iron-sulfur protein YnfG, allowing the reduction of selenate to selenite (Theisen & Yee, 2014).

The Gram-negative, strictly anaerobic bacterial strain *Sulfurospirillum barnesii* SES-3 was isolated from a freshwater marsh in the Stillwater Wildlife Management Area of western Nevada (USA), using a selenate respiration



enrichment culture (Oremland *et al.*, 1994). This strain was shown to convert selenate to selenite by dissimilative reduction under anaerobic conditions coupled with the oxidation of lactate to acetate and CO<sub>2</sub>. No growth was observed on selenite, but cell suspensions readily reduced selenite to Se<sup>0</sup> with a final conversion of ~5% of the initial selenate. *S. barnesii* SES-3 can therefore achieve the complete reduction of selenate to Se<sup>0</sup> and can utilize selenate as final electron acceptor to support growth (Stolz *et al.*, 1999).

Four different species of *Proteobacteria* capable of dissimilatory selenate reduction were isolated by selenate respiration enrichment cultures from a variety of sources, including sediments from three different water bodies in Chennai (India) and a tidal estuary in New Jersey (USA) (Narasingarao & Häggblom, 2007a). *Pelobacter seleniigenes* KM<sup>T</sup> is a strict anaerobe isolated from Kearny Marsh, a New Jersey wetland system. It was classified as a novel species of the class  $\delta$ -*Proteobacteria* due to unique physiological and taxonomic characteristics (Narasingarao & Häggblom, 2007b). The stoichiometric respiration of selenate to Se<sup>0</sup> involves the use of acetate as the electron donor and carbon source. Selenate reduction is followed by a transient accumulation of selenite, which is eventually reduced to Se<sup>0</sup>, resulting in the production of a bright red precipitate. The utilization of 5.4 mM acetate was accompanied by the reduction of 7.3 mM selenate to 4.6 mM selenite and 2.7 mM Se<sup>0</sup>. Interestingly, this strain can also ferment short-chain organic acids, such as pyruvate, citrate and lactate. Selenate-respiring cultures produce abundant Se<sup>0</sup> granules which are closely associated with the cells (Narasingarao & Häggblom, 2007b). *Ferrimonas futtsuensis* FUT3661<sup>T</sup> and *Ferrimonas kyonanensis* Asr22-7<sup>T</sup> are facultative anaerobic, selenate-reducing chemo-organotrophs of the class  $\gamma$ -*Proteobacteria*, isolated from sediments and littleneck clams (*Ruditapes philippinarum*), respectively, collected from the coast of Tokyo Bay (Japan). They can both reduce selenate by using it as a final electron acceptor coupled with lactate oxidation, resulting in the appearance of orange-to-red precipitates representing insoluble Se<sup>0</sup> (Nakagawa *et al.*, 2006). *Sedimenticola selenatireducens* AK4OH1 is a strict anaerobe isolated from estuarine sediments. The reduction of selenate is followed by the stoichiometric accumulation of selenite and the formation of red colonies due to the precipitation of Se<sup>0</sup>. This strain has the unique ability to oxidize aromatic acids such as benzoate, 4-hydroxybenzoate and 3-hydroxybenzoate coupled to selenate respiration (Narasingarao & Häggblom, 2006).

*Bacillus selenatarsenatis* SF-1 is a Gram-positive species that was isolated from Se-rich sediment collected in an effluent drain that had received contaminated discharge from a glass-manufacturing plant (Fujita *et al.*, 1997). Also this strain uses selenate as a terminal electron acceptor coupled with the oxidation of lactate (Kuroda *et al.*, 2011; Yamamura *et al.*, 2007). Selenate is reduced to Se<sup>0</sup> through the intermediate selenite (Fujita *et al.*, 1997; Kashiwa *et al.*, 2000). The strain can reduce selenate at concentrations up to 20 mM, but the reduction of selenate to

selenite is faster than the subsequent reduction of selenite to  $\text{Se}^0$  (Kashiwa *et al.*, 2000). This strain can reduce nitrate to nitrite and arsenate to arsenite under anaerobic conditions as well. In a laboratory-scale continuous bioreactor, the strain removed selenate from synthetic wastewater (41.8 mg Se/L) in the presence of excess lactate as the carbon and energy source. The bioreactor was operated with a cell retention time between 2.2 and 95.2 h. At short cell retention times selenate was removed, but accumulation of selenite was observed. At long cell retention times soluble selenium was reduced to  $\text{Se}^0$  (Fujita *et al.*, 2002).

The selenate reductase SrdBCA from *Bacillus selanatarsenatis* SF-1 has been isolated and characterized as a membrane-bound, trimeric, molybdopterin-containing oxidoreductase (Kuroda *et al.*, 2011). The mechanism of selenate reduction to selenite involves the shunting of an electron pool from the quinone  $\text{QH}_2$  to selenate via the SrdC, SrdB and SrdA subunits.  $\text{QH}_2$  is oxidized to quinone by SrdC, releasing two protons outside the cell membrane and transferring two electrons to SrdB. These electrons pass through the [4Fe-4S] clusters of SrdB and transfer to the [4Fe-4S] cluster of SrdA, and selenate then receives the electrons via the molybdenum cofactor.

*Bacillus arsenicoselenatis* strain E1H is a strictly anaerobic, spore-forming bacterium isolated from the anoxic muds of the alkaline, hypersaline, and arsenic-rich Mono Lake in California (USA). It converts arsenate to arsenite and selenate to selenite with the concomitant oxidation of lactate to acetate and  $\text{CO}_2$  (Switzer-Blum *et al.*, 1998). Similarly, *Selenihalanaerobacter shriftii* DSSe-1 is an obligate anaerobic halophilic bacterium from the Dead Sea that can grow via the respiration of selenate to selenite plus  $\text{Se}^0$  and the concomitant oxidation of glycerol or glucose to acetate and  $\text{CO}_2$  (Blum *et al.*, 2001). *Desulfuribacillus stibiiarsenatis* MLFW-2<sup>T</sup>, isolated from anoxic sediments collected from the drainage area of a geothermal spring near Mono Lake, can grow anaerobically by using both selenate and selenite as final electron acceptors and using lactate, pyruvate, formate or  $\text{H}_2$  as electron donors (Abin & Hollibaugh, 2017).

### 3.2.2 Bacterial selenite respiration

Although selenite respiration plays an important role in the Se biogeochemical cycle, it has received much less attention than selenate respiration. Three bacterial strains have been shown to reduce selenite to  $\text{Se}^0$  under anaerobic conditions, using selenite as the final electron acceptor for respiration (Table 3.1): *Bacillus selenitireducens* MLS10 (Switzer-Blum *et al.*, 1998), *Bacillus beveridgei* MLTeJB (Baesman *et al.*, 2009) and *Desulfurispirillum indicum* S5 (Rauschenbach *et al.*, 2011). *Bacillus selenitireducens* MLS10 is a Gram-positive haloalkaliphilic bacillus isolated from Mono Lake, and was the first bacterial strain shown to grow rapidly via the respiration of selenite (Switzer-Blum *et al.*, 1998). A putative respiratory selenite reductase (Srr) was characterized by

combined biochemical, genomic and proteomic analysis, identifying the protein as a member of the complex iron–sulfur molybdoenzyme (CISM) family (Wells *et al.*, 2019). The 80 kDa catalytic subunit (SrrA) is a periplasmic complex with one putative 4Fe–4S binding site and specificity for selenite as an electron acceptor, with no activity in the presence of arsenate, selenate, or thiosulfate. The  $K_m$  for selenite was  $145 (\pm 53) \mu\text{M}$ , much higher than the values reported for other selenium oxidoreductases. Genomic analysis revealed the presence of an operon containing six genes (*srrE*, *srrA*, *srrB*, *srrC*, *srrD*, and *srrF*). In addition to the catalytic subunit SrrA, the operon coded for a small electron transfer subunit (SrrB, 17.7 kDa) with four putative 4Fe–4S binding sites, an anchoring subunit (SrrC, 43 kDa), a chaperone (SrrD, 24 kDa) and two rhodanese domain-containing proteins (SrrE, 38 kDa; and SrrF, 45.6 kDa).

*Bacillus beveridgei* MLTeJB is a facultative anaerobe isolated from Mono Lake sediment by a tellurite enriched culture. This strain can use multiple electron acceptors to grow under anaerobic conditions, including tellurite, tellurate, selenite, selenate, nitrite, nitrate and arsenate. The strain achieves the complete reduction of selenite to  $\text{Se}^0$  plus selenide over an incubation period of 25 days, coupling selenite reduction with lactate oxidation to acetate and formate (Baesman *et al.*, 2009).

*Desulfurispirillum indicum* S5<sup>T</sup> is a strictly anaerobic strain isolated from the sediment of an estuarine canal in Chennai (India), based on its ability to reduce selenate and selenite to  $\text{Se}^0$ . In addition to Se oxyanions, this strain also achieves the dissimilatory reduction of arsenate and nitrate coupled with the oxidation of pyruvate, lactate and acetate (Rauschenbach *et al.*, 2011).

### 3.3 STRATEGIES FOR THE DETOXIFICATION OF SELENIUM OXYANIONS IN BACTERIA

Reduction chemistry may provide a basic level of resistance to particular elements and compounds in the environment (Avazeri *et al.*, 1997). Indeed, the most widespread mechanism of selenate/selenite detoxification is its reduction to insoluble  $\text{Se}^0$  and subsequent precipitation, producing extracellular or intracellular particles that may be deposited in the cytoplasm or on the cell wall or membrane to form Se nanostructures (Zannoni *et al.*, 2008). This process occurs under both aerobic and anaerobic conditions and is generally more rapid for selenite than selenate.

The reduction of selenate/selenite by bacteria may involve (1) specific or non-specific enzymes, mainly periplasmic or cytosolic oxidoreductases that function under anaerobic or aerobic growth conditions; (2) reaction with thiols via the so-called Painter-type reaction; and (3) interactions with siderophores such as pyridine-2,6-bisthiocarboxylic acid (PDTC).

### 3.3.1 Enzymatic detoxification

Various enzymatic systems may be involved in the reduction of selenate/selenite to  $\text{Se}^0$ , including sulfate, sulfite, nitrate, nitrite and fumarate reductases. For example, a nitrite reductase may be responsible for selenite reduction under anaerobic conditions in *T. selenatis* AX<sup>T</sup>, because mutants lacking this activity are unable to reduce either nitrite or selenite (DeMoll-Decker & Macy, 1993). Similarly, *Rhizobium sullae* HCNT1 was shown to reduce millimolar concentrations of selenite using a copper-containing nitrite reductase (Nir) (Basaglia *et al.*, 2007). The periplasmic nitrate reductase NapA or the cytoplasmic nitrate reductases NarGHIJ and NarZUWV may be responsible for the reduction of selenate to selenite in *Escherichia coli* (Avazeri *et al.*, 1997).

*Shewanella oneidensis* MR-1 possesses multiple respiration reductases and can therefore reduce a wide range of electron acceptors. By exploiting its broad respiratory ability, MR-1 can survive in different environments and shows high resistance to different toxic compounds. *S. oneidensis* MR-1 mutants lacking different reductase activities were tested for their ability to reduce selenite. This revealed that fumarate reductase (FccA) is involved in selenite reduction under anaerobic conditions. The authors tested mutants lacking periplasmic terminal reductases, namely nitrate reductase (NapA), nitrite reductase (NrfA) or fumarate reductase (FccA), as well as mutants deficient for individual genes encoding Mtr proteins, the periplasmic mediators of anaerobic extracellular respiration (Li *et al.*, 2014). The analysis of  $\Delta napB$  and  $\Delta nrfA$  mutants revealed that neither nitrate reductase nor nitrite reductase contributed to selenite reduction in *S. oneidensis* MR-1, nor did the extracellular respiratory proteins. Conversely, selenite reduction by mutant  $\Delta fccA$  was severely suppressed, with a 60% decrease in activity within the initial 12 h of selenite exposure. The cytoplasmic membrane-anchored cytochrome CymA was shown to bridge electron transfer from the quinone pool to several respiratory reductases during anaerobic respiration, including the fumarate reductase FccA. The authors therefore proposed a mechanism in which FccA catalyzes selenite reduction and CymA shunts electrons from the quinol pool to FccA (Li *et al.*, 2014). It seems that anaerobic selenite reduction in *S. oneidensis* MR-1 does not support growth, but is instead a mechanism to detoxify the bacterial environment to prevent selenite uptake (Li *et al.*, 2014; Nancharaiyah & Lens, 2015). The reduction of selenite under anaerobic conditions not coupled to growth has also been reported for *Veilonella atypica*. In this case, the formation of  $\text{Se}^0$  and selenide was observed in bacterial cultures exposed to selenite and supplied with the extracellular electron shuttle anthraquinone-2,6-disulphonate (AQDS), suggesting that selenite reduction was achieved via a hydrogenase-coupled reduction, mediated by ferredoxin (Pearce *et al.*, 2009).

In the case of *Alishewanella* sp. WH16-1, a facultative anaerobe from mining soil, a novel aerobic selenite reductase was identified, named chromate selenite

reductase flavoenzyme (CrsF). This flavoprotein, with 37% identity to *E. coli* chromate reductase (ChrR), is able to reduce selenite or chromate *in vitro*, as well as sulfate and ferric anions, using NADPH as a cofactor (Xia *et al.*, 2018). An interesting study of selenate and selenite reduction under aerobic conditions in the obligate aerobic bacterium *Comamonas testotsteroni* S44 revealed evidence that selenate is reduced via the sulfate reduction pathway (Tan *et al.*, 2018). The strain was first isolated from a metal-polluted site and was shown to transform both selenite and selenate to  $\text{Se}^0$ , resulting in the formation of intracellular Se nanostructures (Zheng *et al.*, 2014). Transposon mutagenesis experiments revealed that the Cys regulon transcriptional activator CysB (involved in sulfur metabolism and responsible for regulating cysteine anabolism) is involved in the reduction of selenate to  $\text{Se}^0$  (Tan *et al.*, 2018). The authors also identified an aerobic selenite reductase (SerT), a member of the molybdenum-containing sulfite oxidase family localized in the periplasmic space. This concurred with electron microscopy results showing the presence of Se nanoparticles in the cytoplasm when *C. testotsteroni* was exposed to selenate, but in the periplasm when it was exposed to selenite (Tan *et al.*, 2018).

Under anaerobic conditions, several bacterial strains have been shown to reduce both selenate and selenite in the cytoplasm or periplasm without using them as final electron acceptors for respiration, including *Desulfovibrio desulfuricans* DSM1924 (Tomei *et al.*, 1995) and *Wolinella succinogenes* (Tomei *et al.*, 1992). *D. desulfuricans* DSM1924 can grow in the presence of 10 mM selenate or 0.1 mM selenite by using sulfate or fumarate as an electron acceptor and formate as an electron donor. It can reduce both selenate and selenite to  $\text{Se}^0$ , resulting in the precipitation of Se deposits in the periplasm and cytoplasm, respectively. *Wolinella succinogenes* can grow in the presence of 10 mM selenate or 0.1 mM selenite by using fumarate as an electron acceptor and formate as an electron donor, but only after an adaptation step with sublethal doses of Se oxyanions.

### 3.3.2 Thiol driven reactions

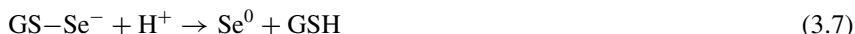
#### 3.3.2.1 Reaction mechanisms

The reduction of selenite to  $\text{Se}^0$  is mediated by reaction with thiols. Painter (1941) studied the chemical reaction of selenite with thiol groups and was the first to demonstrate the formation of selenium disulfides (RS-Se-SR) through the following reaction (Equation. 3.5):

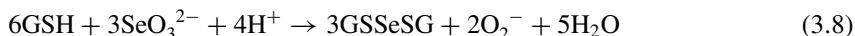


Ganther (1971) investigated the reaction of selenite with the thiol-containing molecule glutathione (GSH), the most abundant thiol found in eukaryotic cells, cyanobacteria, and the bacterial phylum Proteobacteria. The reaction of selenite with GSH was shown to form an intermediate (Se-diglutathione) that provides an excellent substrate for glutathione reductase, with  $K_m$  and  $V_{\text{max}}$  values

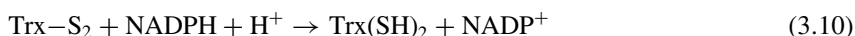
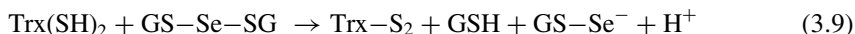
comparable to those of GSH itself. The reaction of Se-diglutathione with NAD(P)H glutathione reductase leads to the formation of a highly unstable product, the selenium persulfide anion  $\text{GS-Se}^-$  (Equation 3.6). Ganther (1971) also suggested that the persulfide anion reverted quickly to elemental  $\text{Se}^0$  as shown in (Equation 3.7):



Kessi and Hanselmann (2004) proposed a modification of (Equation 3.5) which leads to the formation of a superoxide anion (Equation 3.8). This was based on the comparison of the abiotic (chemical) reduction of selenite by glutathione and a reaction mediated by *Rhodospirillum rubrum* and *E. coli* when exposed to selenite.



Superoxide anions are removed from biological systems by superoxide dismutase (SOD) and catalase to protect cells from oxidative stress, explaining the induction of two types of SOD in *E. coli* grown in the presence of selenite (Bébién *et al.*, 2002). Thiol-containing biomolecules other than glutathione may also be involved, such as the reduction of selenite and Se-diglutathione by the thioredoxin system of *E. coli* (Björnstedt *et al.*, 1992). The authors proposed that Se-diglutathione is reduced by thioredoxin that is in turn reduced by NADPH-dependent thioredoxin reductase to regenerate reduced thioredoxin as shown below (Equations 3.9 and 3.10) (Björnstedt *et al.*, 1992; Kumar *et al.*, 1992):



The selenite reaction mechanism proposed by Kessi and Hanselman (2004) may therefore involve three steps: an abiotic step in which selenite is transformed into Se-diglutathione with the concomitant release of reactive oxygen species (ROS) as reported in (Equation 3.8), an enzymatic step in which NAD(P)H-dependent reductases such as glutathione or thioredoxin reductases lead to the production of the unstable persulfide anion via (Equation 3.6), and finally the abiotic formation of  $\text{Se}^0$  via (Equation 3.7). The initial reducing power is thereby restored by regenerating six molecules of GSH.

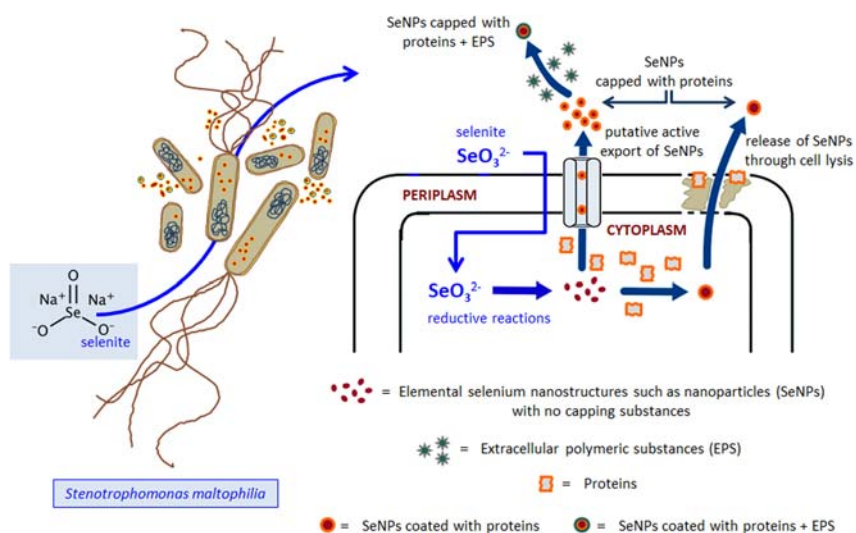
### 3.3.2.2 Microbial strategies for thiol based Se detoxification

#### 3.3.2.2.1 Gram negative bacteria

Several bacterial strains have been identified and characterized for their ability to reduce selenite into  $\text{Se}^0$  under either anaerobic or aerobic conditions using thiols. A well-documented case is *Stenotrophomonas maltophilia* SeITE02, a bacterial isolate obtained from the rhizosphere of the Se-hyperaccumulating legume *Astragalus bisulcatus* grown on a seleniferous soil. This strain can survive

exposure to 50 mM selenite and can reduce up to 2.0 mM selenite in 48 hours after a conditioning step, with the concomitant formation of  $\text{Se}^0$  nanostructures (Di Gregorio *et al.*, 2005). These structures were initially localized in the cytoplasmic fraction, but later also accumulated in the extracellular space (Di Gregorio *et al.*, 2005; Lampis *et al.*, 2017).

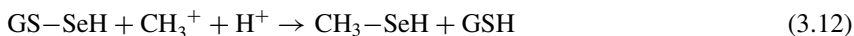
It remains largely unclear how  $\text{Se}^0$  nanostructures reach the extracellular space. One potential release mechanism is cell lysis, because  $\text{Se}^0$  nanostructures have been detected by electron microscopy in the spent medium close to cell ghosts (Di Gregorio *et al.*, 2005; Lampis *et al.*, 2017). This hypothesis was also proposed for *Desulfovibrio desulfuricans* exposed to selenate and selenite (Tomei *et al.*, 1995). More recently, *S. maltophilia* SeITE02 cells grown in defined medium supplied with glucose or pyruvate as carbon sources and exposed to selenite were found to generate membrane vesicles that surrounded the extracellular  $\text{Se}^0$  nanostructures, indicating that such vesicles might play a key role in the excretion of  $\text{Se}^0$  nanostructures into the extracellular environment (Piacenza *et al.*, 2018) (Figure 3.2). The export of  $\text{Se}^0$  nanostructures has already been proposed for other bacterial strains, including *R. rubrum* and *T. selenatis* AX<sup>T</sup> (Debieux *et al.*, 2011; Kessi *et al.*, 1999).



**Figure 3.2** Selenite transformation reactions in *Stenotrophomonas maltophilia* SeITE02. Once inside the cell, selenite is reduced in the cytoplasmic compartment to form  $\text{Se}^0$  nanostructures such as nanoparticles (SeNPs). These are exported via a release mechanism based on cell lysis or by an unknown active process.  $\text{Se}^0$  nanostructures are surrounded by an organic coating composed mostly of proteins and/or extracellular polymeric substances (EPS).

Combined biochemical, physiological and proteomic analysis revealed that *S. maltophilia* SeITE02 does not use nitrite reductase for the reduction of selenite, but there is evidence these two oxyanions may share the same transport system (Antonioli *et al.*, 2007). Moreover, *in vitro* enzymatic assays demonstrated that selenite reduction occurs in the cytoplasmic fraction and the enzyme is NAD(P)H-dependent. Afterwards, growth tests in the presence of the GSH synthesis inhibitor *S-n*-butyl homocysteine sulfoximine suggested that GSH is involved in the first-stage response of the cells to selenite exposure. Proteomic analysis revealed that the main functional classes of proteins upregulated by exposure to either selenite or nitrite are those related to damaged-protein catabolism, DNA metabolism and cell division, as well as oxidative stress responses. Interestingly, selenite seems to strongly induce the expression of at least two different GSH-related enzymes (glutamate-cysteine ligase and GSH synthetase) that are responsible for the biosynthesis of GSH in prokaryotes. Selenite reduction via glutathione was also proposed for *Ochrobactrum* sp. MPV1, isolated from an arsenopyrites dump. This strain can survive exposure to 80 mM selenite and accumulates intracellular Se<sup>0</sup> nanostructures mainly in the cytoplasm (Zonaro *et al.*, 2017).

The mechanism of selenite reduction has also been investigated in methane-oxidizing bacteria such as *Methylococcus capsulatus* (Bath), which are capable of the methane-driven conversion of selenite to Se<sup>0</sup> nanoparticles and methylated selenium species (Eswayah *et al.*, 2017). Chromatographic and spectroscopic analyses of *M. capsulatus* (Bath) cells exposed to selenite in the presence of methane to allow the formation of Se<sup>0</sup> nanoparticles, indicated that methylselenol (CH<sub>3</sub>SeH) is the key intermediate in the reduction of selenite into methylated Se (Equations 3.11 through 3.13) and Se-S species, as well as Se<sup>0</sup> nanostructures (Eswayah *et al.*, 2019):



### 3.3.2.2.2 Gram positive bacteria

A different mechanism of selenite reduction may occur in Gram-positive bacteria, which are unable to synthesize glutathione but can tolerate high levels of selenite. *Bacillus subtilis* was shown to reduce selenite to Se<sup>0</sup>, which precipitated as Se granules between the cell wall and plasma membrane (Garbisu *et al.*, 1995). The authors confirmed that selenite reduction involves an inducible detoxification system rather than dissimilative electron transport. The reduction of selenite in



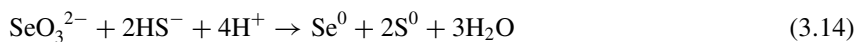
*B. subtilis* not only facilitates adaptation to high selenite concentrations but also induces a morphological change from rod-like to rounded cells. The mechanism of reduction appears to involve a dithiol system because thioredoxin and NADPH-thioredoxin reductase levels increased following exposure to selenite (Garbisu *et al.*, 1999).

The *Bacillus mycoides* strain SeITE01, isolated from the rhizosphere of *Astragalus bisulcatus* (Vallini *et al.*, 2005) completely reduces 2.0 mM selenite in the liquid culture after 24 h under aerobic conditions (Lampis *et al.*, 2014). Selenite reduction was associated with the formation of intracellular and extracellular Se<sup>0</sup> nanostructures, and because there was evidence of selenite reduction in the membrane protein fraction and spent medium, the authors proposed that the corresponding enzymes were not only found inside the cells but were also secreted to the extracellular space (Lampis *et al.*, 2014).

Selenite reduction may also have been facilitated by other thiol-rich molecules such as bacillithiol (BSH), a major low-molecular-weight thiol that plays a significant role in the cytosolic thiol-redox chemistry of Gram-positive bacteria together with thioredoxin (Gaballa *et al.*, 2010). BSH-synthesizing bacteria may contain enzymes analogous to those found in GSH-containing species, with bacilliredoxin (Brx) instead of glutaredoxin (Grx). In *B. mycoides* SeITE01 a Brx-like protein may play a similar role to Grx in an analogous pathway to the GSH system found in Gram-negative bacteria (Lampis *et al.*, 2014).

### 3.3.2.2.3 Reaction with sulfide

Selenite can also undergo abiotic reactions with biogenic sulfides to yield elemental Se and S (Equation 3.14). This has been observed in sulfate-reducing bacteria such as *Desulfomicrobium norvegicum*, where the reduction of sulfate is linked to the concomitant extracellular precipitation of elemental S and Se in cell cultures exposed to selenite (Hockin & Gadd, 2003).



## 3.3.3 Siderophore driven detoxification

The reduction of selenite to Se<sup>0</sup> as a detoxification mechanism in *Pseudomonas stutzeri* KC is promoted by the siderophore PDTC, a broad-range metal chelator (Zawadzka *et al.*, 2006). Siderophores are iron-specific chelators excreted by microorganisms under iron-limiting conditions as a part of an iron acquisition system. The authors proposed that selenite could be reduced after binding to PDTC or its hydrolysis product dipicolinic acid-pyridine-2,6-bis(carboxylic acid) (DPA). The production and excretion of PDTC facilitates the extracellular reduction of metalloids and thus acts as an environmental detoxification mechanism that prevents the uptake of selenite into cells.

### 3.4 BIOTRANSFORMATION OF SELENIUM OXYANIONS BY ARCHAEA

A list of archaeal species associated with the aerobic or anaerobic reduction of Se oxyanions is provided in Table 3.2. One of the most interesting examples of selenate-respiring *Archaea* species is *Pyrobaculum arsenaticum* PZ6, a hyper-thermophilic, strictly anaerobic, facultative organotrophic strain isolated from a hot spring at Pisciarelli Solfatara (Naples, Italy). This strain can grow organotrophically in the presence of selenate, arsenate or elemental sulfur as inorganic electron acceptors (Huber *et al.*, 2000). Similarly, *Pyrobaculum ferrireducens* 1860(T) is a hyperthermophilic, anaerobic archaeon isolated from a terrestrial hot spring at Uzon Caldera, Kronotsky Nature Reserve (Kamchatka, Russia) (Slobodkina *et al.*, 2015). It can grow anaerobically using Fe(III), nitrate, arsenate, selenate and selenite as final electron acceptors. *Pyrobaculum aerophilum* is a hyperthermophilic strain belonging to the archaeon phylum *Crenarchaeota*. This strain is capable of chemolitho-autotrophic growth with selenate as the electron acceptor in the presence of H<sub>2</sub>, and can also grow by the respiration of either selenate or selenite under organotrophic conditions in a basal salt medium supplied with yeast extract (Huber *et al.*, 2000; Völkl *et al.*, 1993). Interestingly, the reduction of selenite resulted in the formation of black colored Se<sup>0</sup> precipitates.

More recently, a microbial consortium capable of methane-dependent selenate reduction was enriched in a membrane biofilm batch reactor. Metagenomic analysis revealed that the consortium was mainly composed of methanotrophic archaeons belonging to the genus *Methanosarcina* and type II methanotrophic bacteria belonging to the genus *Methylocystis*, which carried out selenate reduction along with methane oxidation (Shi *et al.*, 2020). This agrees with recent insights into methane-metabolizing archaeons that either produce or consume methane (Evans *et al.*, 2019).

### 3.5 FUNGAL TRANSFORMATION OF SELENIUM OXYANIONS

#### 3.5.1 Introduction

The aerobic reduction of selenite and selenate in the absence of respiration or assimilation reactions is used by many bacteria as a detoxification mechanism (Doran, 1982; Eswayah *et al.*, 2016; Gadd, 1993; Soudi *et al.*, 2009). This has considerable potential as a cost-effective approach for the bioremediation of seleniferous environments (Javed *et al.*, 2016; Maltman & Yurkov, 2018). Nevertheless, the ability to aerobically reduce selenite and/or selenate to elemental selenium (Se<sup>0</sup>), or even to selenide (Se<sup>2-</sup>) in the form of volatile methylated compounds such as dimethyl selenide (DMSe: CH<sub>3</sub>SeCH<sub>3</sub>), dimethyl

Table 3.2 Archaea capable of the selective reduction of selenium oxyanions.

Species	Taxonomic Classification	Reduction of Se Oxyanions	Reduction Conditions	Formation of Elemental Selenium Nano-structures	Reference
<i>Halobacterium</i> sp. SP1	<i>Euryarchaeota</i> , <i>Halobacteriales</i> (extreme halophiles)	10 mM selenite	AE	Yes – as amorphous red elemental Se precipitate	Naik <i>et al.</i> , 2017
<i>Halococcus salifodinae</i> BK18	<i>Euryarchaeota</i> , <i>Halobacteriales</i> (extreme halophiles)	2 mM selenite	AE	Yes – as amorphous red elemental Se nanoparticles	Srivastava <i>et al.</i> , 2014.
<i>Halogetometricum borinquense</i> E118	<i>Euryarchaeota</i> , <i>Halobacteriales</i> (extreme halophiles)	5 mM selenite	AE	Yes – intracellular amorphous red elemental Se nanoparticles	Abdollahnia <i>et al.</i> , 2020
<i>Halorubrum xinjiangense</i>	<i>Euryarchaeota</i> , <i>Halobacteriales</i> (extreme halophiles)	25 mM selenite	AE	Yes – as amorphous red elemental Se nanoparticles	Güven <i>et al.</i> , 2013
<i>Methanosarcina</i> sp.	<i>Euryarchaeota</i> , <i>Methanosarcinales</i> (methanogens)	~30 mg Se/L as selenate	ANA	Progressive disappearance of $\text{SeO}_4^{2-}$ via transient $\text{SeO}_3^{2-}$ accumulation due to the activity of a <i>Methanosarcina</i> / <i>Methylocystis</i> consortium in bioreactor; Formation of amorphous red $\text{Se}^0$ starting from selenite reduction	Shi <i>et al.</i> , 2020

(Continued)

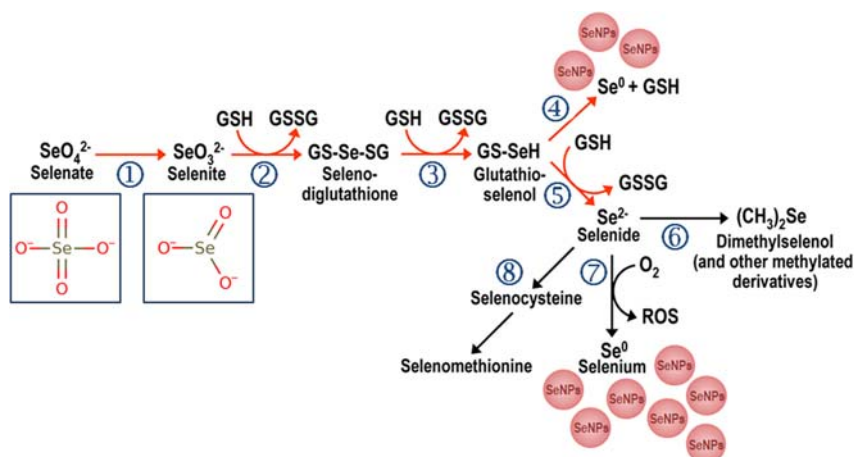
Table 3.2 Archaea capable of the selective reduction of selenium oxyanions (Continued).

Species	Taxonomic Classification	Reduction of Se Oxyanions	Reduction Conditions	Formation of Elemental Selenium Nano-structures	Reference
<i>Pyrobaculum aerophilum</i>	<i>Crenarchaeota</i> , <i>Thermoproteales</i> (hyperthermophiles)	Organotrophic respiration of either selenate or selenite as final electron acceptors Chemolitho-autotrophic growth with selenate as electron acceptor in the presence of H <sub>2</sub>	ANA	Yes – as hexagonal gray elemental Se; only during growth on selenite	Huber <i>et al.</i> , 2000
<i>Pyrobaculum arsenaticum</i>	<i>Crenarchaeota</i> , <i>Thermoproteales</i> (hyperthermophiles)	Anaerobic respiration of selenate	ANA	Yes – as a precipitate of hexagonal gray elemental Se	Huber <i>et al.</i> , 2000
<i>Pyrobaculum ferrireducens</i> 1860 <sup>T</sup>	<i>Crenarchaeota</i> , <i>Thermoproteales</i> (hyperthermophiles)	Anaerobic respiration of 10 mM selenite or 10 mM selenate	ANA	Yes – red precipitate with selenite, black precipitate with selenate	Slobodkina <i>et al.</i> , 2015

Reduction: AE (aerobic); ANA (anaerobic).

selenyl sulfide (DMS<sub>2</sub>Se: CH<sub>3</sub>SeSCH<sub>3</sub>), and dimethyl diselenide (DMDSe: CH<sub>3</sub>SeSeCH<sub>3</sub>), is not unique to bacteria (Chasteen & Bentley, 2003; Herrero & Wellinger, 2015) (Figure 3.3). More recently, this property has been observed in a broad range of fungi (Morley *et al.*, 1996; Rosenfeld *et al.*, 2017; Sinharoy & Lens, 2020), including yeasts (Falcone & Nickerson, 1963; Kieliszek *et al.*, 2015; Soudi, 2003), filamentous fungi, and higher fungi (i.e., mushrooms) within the members of *Zygomycota* (Gharieb *et al.*, 1995), *Ascomycota* (Ramadan *et al.*, 1988; Sarkar *et al.*, 2011), and *Basidiomycota* (Espinosa-Ortiz *et al.*, 2015a; Vetchinkina *et al.*, 2016). A summary list of fungal species capable of selenite and/or selenate reduction is presented in Table 3.3.

Se tolerance and detoxification by fungi mainly involves the reduction of inorganic Se to less toxic and volatile derivatives such as DMS<sub>2</sub>Se (Gadd, 1993), or



**Figure 3.3** Pathways for the metabolic reduction of inorganic selenium (Se) and its conversion into organic species or the elemental form (Se<sup>0</sup>) in *Saccharomyces cerevisiae* and other yeasts. Reductive reactions are shown as red arrows. Reaction 1 represents multiple reactions involving ATP sulfurylase and other enzymes from the initial steps of the sulfate assimilation pathway. Reactions 2–5 are non-enzymatic and result in the net conversion of reduced glutathione (GSH) to oxidized glutathione as glutathione disulfide (GSSG). Selenide can give rise to volatile methylated forms via reaction 6. Reaction 7 is also non-enzymatic, and results in the formation of diverse reactive oxygen species (ROS) along with Se<sup>0</sup> nanoparticles (which are also generated by reaction 4). In reaction 8, the Se-containing amino acids selenocysteine and selenomethionine are formed and incorporated into selenoproteins [adapted from Herrero & Wellinger, 2015].

**Table 3.3** Fungal species capable of selenite and/or selenate reduction, associated with the accumulation of red amorphous Se<sup>0</sup> particulate matter.

Fungal Species	Selenite Reduction	Selenate Reduction	Reduction Products	Reference
<b>FILAMENTOUS AND HIGHER FUNGI</b>				
<i>Acremonium strictum</i> [A]	+	+	Se <sup>0</sup> nanoparticles; Se methylated volatile compounds	Rosenfeld <i>et al.</i> , 2017
<i>Agaricus arvensis</i> [B]	+		Spherical Se <sup>0</sup> nanoparticles	Vetchinkina <i>et al.</i> , 2019
<i>Agaricus bisporus</i> [B]	+		Spherical Se <sup>0</sup> nanoparticles	Vetchinkina <i>et al.</i> , 2019
<i>Alternaria alternata</i> [A]	+	+	Se <sup>0</sup> nanorods; Se <sup>0</sup> nanoparticles; Se methylated volatile compounds	Rosenfeld <i>et al.</i> , 2017; Sarkar <i>et al.</i> , 2012
<i>Aspergillus clavatus</i> [A]		+	Negligible zero-valent particles; Se methylated volatile compounds	Urik <i>et al.</i> , 2016
<i>Aspergillus funiculosus</i> [A]	+		Needle-like crystals of Se <sup>0</sup>	Gharieb <i>et al.</i> , 1995
<i>Aspergillus niger</i> [A]	+		Amorphous red elemental Se <sup>0</sup>	Gharieb <i>et al.</i> , 1995
<i>Aspergillus oryzae</i> [A]	+		Se <sup>0</sup> nanoparticles	Kimura <i>et al.</i> , 2014
<i>Aspergillus parasiticus</i> [A]	+	+	Se <sup>0</sup> nanoparticles	Moss <i>et al.</i> , 1987
<i>Aspergillus</i> sp. J2 [A]	+		Se <sup>0</sup> nanoparticles	Li <i>et al.</i> , 2018
<i>Coriolus versicolor</i> [B]	+		Amorphous red elemental Se <sup>0</sup>	Gharieb <i>et al.</i> , 1995
<i>Flammulina velutipes</i> [B]	+		Se <sup>0</sup> nanoparticles	Wang <i>et al.</i> , 2016
<i>Fusarium</i> sp. [A]	+	+	Red elemental Se <sup>0</sup> particulate; Needle-like crystals of Se <sup>0</sup>	Gharieb <i>et al.</i> , 1995; Ramadan <i>et al.</i> , 1988
<i>Ganoderma lucidum</i> [B]	+		Se <sup>0</sup> nanoparticles	Rosenfeld <i>et al.</i> , 2017
<i>Grifolia frondosa</i> [B]	+		Se <sup>0</sup> nanoparticles	Rosenfeld <i>et al.</i> , 2017
<i>Lentinula edodes</i> [B]	+	+	Se <sup>0</sup> nanoparticles	Vetchinkina <i>et al.</i> , 2013

<i>Mortierella humilis</i> [M]	+		Se <sup>0</sup> nanoparticles	Liang et al., 2019
<i>Mortierella</i> sp. [M]	+	+	Se <sup>0</sup> nanoparticles; Se methylated volatile compounds	Zieve et al., 1985
<i>Mucor hiemalis</i> [Z]	+		Amorphous red elemental Se <sup>0</sup>	Gharieb et al., 1995
<i>Mucor</i> SK [Z]	+		Amorphous red elemental Se <sup>0</sup>	Gharieb et al., 1995
<i>Paraconiothyrium sporulosum</i> [A]	+		Stable Se <sup>0</sup> nanoparticles;	Rosenfeld et al., 2020
<i>Penicillium chrysogenum</i> [A]	+		Organo-selenium (Se <sup>-II</sup> ) compounds	Gharieb et al., 1995
<i>Penicillium funiculosum</i> [A]	+		Amorphous red elemental Se <sup>0</sup>	Gharieb et al., 1995
<i>Phanerochaete chrysosporium</i> [B]	+	+	Se <sup>0</sup> nanoparticles	Espinosa-Ortiz et al., 2015a, b
<i>Phoma glomerata</i> [A]	+		Se <sup>0</sup> nanoparticles	Liang et al., 2019
<i>Plectosphaerella cucumerina</i> [A]	+	+	Se <sup>0</sup> nanoparticles; Se methylated volatile compounds	Rosenfeld et al., 2017
<i>Pleurotus ostreatus</i> [B]	+		Se <sup>0</sup> nanoparticles	Vetchinkina et al., 2016
<i>Pyrenochaeta</i> sp. [A]	+	+	Se <sup>0</sup> nanoparticles; Se methylated volatile compounds	Rosenfeld et al., 2017
<i>Rhizopus arrhizus</i> [M]	+		Amorphous red elemental Se <sup>0</sup>	Gharieb et al., 1995
<i>Stagonospora</i> sp. [A]	+	+	Stable Se <sup>0</sup> nanoparticles;	Rosenfeld et al., 2020
<i>Trichoderma harzianum</i> [A]	+		Organo-selenium (Se <sup>-II</sup> ) compounds	Liang et al., 2019
<i>Trichoderma reesei</i> [A]	+		Se <sup>0</sup> nanoparticles; Se oxide (SeO <sub>2</sub> , downeyite) nanoparticles	Gharieb et al., 1995
			Amorphous red elemental Se <sup>0</sup>	

(Continued)

**Table 3.3** Fungal species capable of selenite and/or selenate reduction, associated with the accumulation of red amorphous Se<sup>0</sup> particulate matter (*Continued*).

Fungal Species	Selenite Reduction	Selenate Reduction	Reduction Products	Reference
<b>YEAST-LIKE FUNGI</b>				
<i>Aureobasidium pullulans</i> [A]	+		Se <sup>0</sup> nanoparticles	Gharieb <i>et al.</i> , 1995; Liang <i>et al.</i> , 2019
<b>YEASTS</b>				
<i>Candida albicans</i> [A]	+		Elemental Se <sup>0</sup> precipitate	Falcone and Nickerson, 1963; Gharieb <i>et al.</i> , 1995
<i>Candida glabrata</i> [A]	+		Amorphous red elemental Se <sup>0</sup>	Gharieb <i>et al.</i> , 1995
<i>Candida humicola</i> [A]	+	+	Se methylated volatile compounds; Elemental Se <sup>0</sup> precipitate	Cox and Alexander, 1974; Herrero and Wellinger, 2015
<i>Candida lipolytica</i> [A]	+		Amorphous red elemental Se <sup>0</sup>	Gharieb <i>et al.</i> , 1995
<i>Candida maltosa</i> [A]		+	Elemental Se <sup>0</sup> precipitate	Golubev and Golubev, 2002
<i>Cryptococcus</i> sp. [B]		+	Elemental Se <sup>0</sup> precipitate	Golubev and Golubev, 2002
<i>Hanseniaspora valbyensis</i> [A]		+	Elemental Se <sup>0</sup> precipitate	Golubev and Golubev, 2002
<i>Kluyveromyces marxianum</i> [A]		+	Elemental Se <sup>0</sup> precipitate	Golubev and Golubev, 2002



<i>Rhodotorula mucilaginosa</i> [B]	+	Se <sup>0</sup> nanoparticles	Ruocco <i>et al.</i> , 2014
<i>Rhodotorula rubra</i> [B]	+	Amorphous red elemental Se <sup>0</sup>	Gharieb <i>et al.</i> , 1995
<i>Saccharomyces cerevisiae</i> [A]	+	Amorphous red elemental Se <sup>0</sup>	Gharieb <i>et al.</i> , 1995; Zhang <i>et al.</i> , 2012
<i>Trichosporon</i> sp. [B]	+	Elemental Se <sup>0</sup> precipitate	Golubev and Golubev, 2002
<i>Yarrowia lipolytica</i> [A]	+	Elemental Se <sup>0</sup> precipitate	Golubev and Golubev, 2002
<i>Zygosaccharomyces rouxii</i> [A]	+	Amorphous red elemental Se <sup>0</sup>	Gharieb <i>et al.</i> , 1995

[A] = Ascomycota; [B] = Basidiomycota; [M] = Mucoromycota; [Z] = Zygomycota.

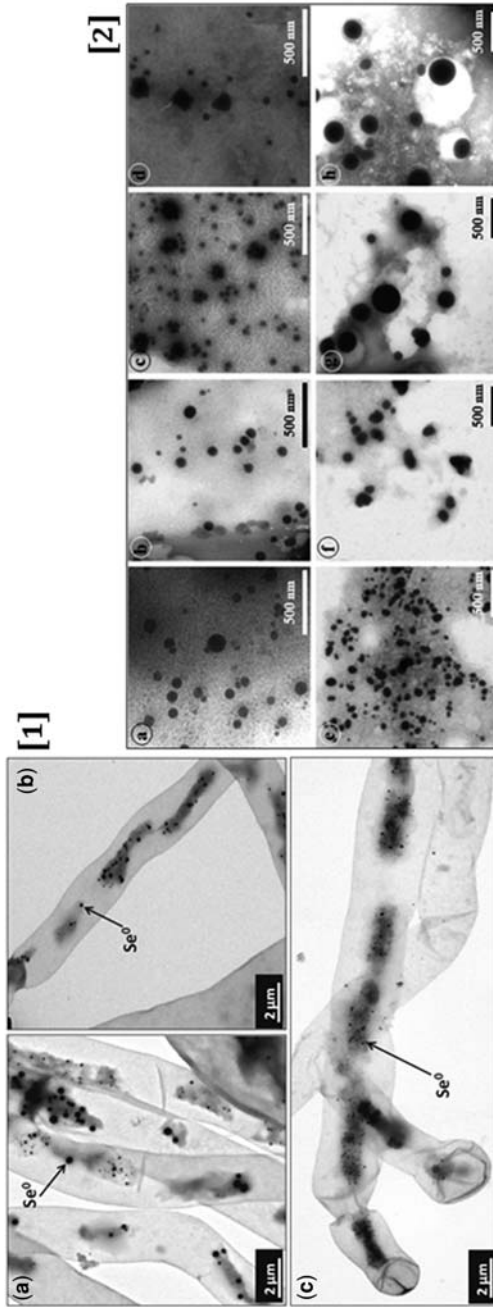
the reduction of Se oxyanions to  $\text{Se}^0$  resulting in the formation of intracellular or extracellular orange-to-red deposits (Gharieb *et al.*, 1995; Konetzka, 1977) (Figure 3.4).

### 3.5.2 Yeasts

A broad review on the tolerance of ascomycetous and basidiomycetous yeasts to selenate was presented by Golubev and Golubev (2002). The ascomycetes *Candida maltosa*, *Hanseniasspora valbyensis*, *Kluyveromyces marxianus* and *Yarrowia lipolytica* show high tolerance to selenate, as do the basidiomycetes *Cryptococcus curvatus*, *Cr. humicola*, and *Trichosporon* spp. A few strains are able to grow at a selenate concentration of 0.1 M, although growth under these conditions is poor. Eventually, these yeasts produce pink-to-red colonies reflecting the reduction of selenate to  $\text{Se}^0$ , whereas the colonies remain colorless in the absence of selenate.

Yeasts were initially tested for their ability to reduce selenite by inoculating them onto MYGP (Malt extract, Yeast extract, Glucose and Peptone) agar supplemented with selenite at concentrations of 0.5–5 mM (Gharieb *et al.*, 1995). Several *Candida* spp. and *Saccharomyces* spp. were able to reduce selenite to  $\text{Se}^0$  at all concentrations, resulting in the formation of pink colonies. In the baker's yeast *Saccharomyces cerevisiae*, Zhang *et al.* (2012) reported the biogenesis of red elemental selenium/protein nanoparticles following the reduction of selenate to  $\text{Se}^0$  in a microaerophilic environment. Other strains, such as *Candida lipolytica* 37-1 and *Rhodotorula rubra* NCYC 797, produced bright red colonies due to their efficient reduction of selenite. Colonies of the polymorphic fungus *Aureobasidium pullulans* were – depending on the  $\text{SeO}_3^{2-}$  concentration – light pink in the presence of 1 mM selenite but red at a higher (5 mM) concentration (Gharieb *et al.*, 1995). The marine yeast strain *Rhodotorula mucilaginosa*-13B provides another clear example of the transformation of selenite into elemental Se in the form of intracellular or extracellular  $\text{Se}^0$  nanoparticles (Ruocco *et al.*, 2014).

An illuminating contribution to the description of the interactions between yeast cells, mostly *S. cerevisiae* and *Candida* spp., and the different chemical species of Se, including Se oxyanions, is provided by Kieliszek *et al.* (2015). Inorganic Se forms such as selenite and selenate enter the yeast cells via promiscuous oxyanion transporters, and are reduced to avoid toxicity. They undergo a series of reduction reactions ultimately leading to the formation of selenide ( $\text{H}_2\text{Se}$ ) using a pathway that, under physiological conditions, involves GSH. In many microbial species, selenide is a common intermediate used for the synthesis of selenoproteins or destined for conversion into methylated forms that are then eliminated through volatilization (Chasteen & Bentley, 2003). Actually, Cox and Alexander (1974) observed the formation of DMSe in cultures of *Candida humicola* supplemented with selenite and selenate. Another possibility is,

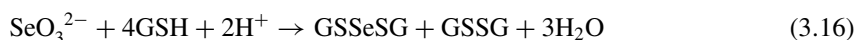


**Figure 3.4** Intracellular and extracellular occurrence of SeNPs in fungi. [1] Transmission electron micrographs of elemental selenium nanoparticles produced in the biomass of the filamentous basidiomycete *P. chrysosporium*. A – distribution of Se<sup>0</sup> particles of different sizes within fungal biomass. B and C – localization of Se<sup>0</sup> particles within fungal biomass [from: [Espinosa-Ortiz et al., 2015a](#)]. [2] TEM of Se<sup>0</sup> (or even SeO<sub>2</sub>) nanoparticles in a number of higher basidiomycetes, nanoparticles produced from Na<sub>2</sub>SeO<sub>3</sub> with extracellular (a–d) and intracellular (e–h) extracts of *L. edodes* (a, e), *P. ostreatus* (b, f), *G. lucidum* (c, g), and *G. frondosa* (d, h). Bars mark 500 nm [from: [Vetchinkina et al., 2018](#)].

however, the reverse oxidation of selenide to  $\text{Se}^0$ , which accumulates inside the cell (Equation 3.15)



It is well known that  $\text{SeO}_3^{2-}$  can react spontaneously with GSH to initially produce selenodiglutathione (GS-Se-SG), (Equation 3.16). In the presence of excess GSH, GSSeSG is further reduced to glutathioselenol (GSSeH), (Equation 3.17). GSSeH either spontaneously dismutates into  $\text{Se}^0$  and GSH, (Equation 3.18), or is further reduced by GSH to yield  $\text{H}_2\text{Se}$  (Equation 3.19). In oxic conditions, selenide is readily re-oxidized by  $\text{O}_2$  into  $\text{Se}^0$  (Equation 3.15) (Cupp-Sutton & Ashby, 2016).



### 3.5.3 Filamentous fungi

Filamentous fungi are also able to reduce selenium oxyanions, resulting in the formation of intracellular or extracellular  $\text{Se}^0$  nanostructures. The pioneering study of Gharieb *et al.* (1995), discussed above for yeast, also revealed that a few species of filamentous fungi were able to carry out the reduction of selenite to  $\text{Se}^0$ . Increasing the selenite concentration from 1 to 10 mM generally led to more severe growth inhibition, although other factors affecting growth included the presence of sulfur compounds and/or ingredients that form selenite complexes in the medium. The species that successfully reduced selenite while growing on Czapek-Dox Agar (CDA) were *Fusarium* sp. and *Trichoderma reesei*, and – to a lesser extent – *Mucor hiemalis* and *Penicillium chrysogenum*, leading to the formation of red-to-orange colonies. Interestingly, electron microscopic images clearly showed that *Fusarium* sp. and *Aspergillus funiculosus* exposed to 50 mM selenite were able to form  $\text{Se}^0$  crystals on the surface of the hyphae. Other species – namely *Aspergillus niger*, *Coriolus versicolor*, *Mucor* SK, and *Rhizopus arrhizus* – were instead able to reduce selenite only on Malt Extract Agar (MEA). These observations suggest that even different culture conditions such as nutrient supply, temperature, pH, and incubation time affect Se metabolism and the final characteristics of the Se nanoparticles. However, the effects of such parameters remain unclear, requiring further investigation for each fungal strain.

Importantly, not all the fungal species tested by Gharieb *et al.* (1995) were able to form  $\text{Se}^0$ . Some of these were able to reduce selenite via other mechanisms, such as methylation and/or the formation of  $\text{Se}^{2-}$  volatiles. Accordingly, the ability to grow on selenite does not necessarily reflect the ability to reduce selenite to  $\text{Se}^0$ . For

example, [Brady et al. \(1996\)](#) showed that *Penicillium* sp. grown aerobically for 2 weeks in the presence of 1 mM selenite releases volatile organic derivatives (probably DMSe) during all four cultivation phases (lag, exponential growth, stationary and decline), whereas the reduction of selenite to amorphous  $\text{Se}^0$  was only observed during the decline phase, as evidenced by the characteristic red color of both the fungal biomass and the culture substrate.

### 3.5.4 Higher fungi (mushrooms)

#### 3.5.4.1 Ascomycetes

A deeper understanding of the role of fungi in the complex reactions characterizing the biogeochemical cycle of selenium has been powerfully reinforced by new insights into the ascomycetes *Paraconiothyrium sporulosum* and *Stagonospora* sp. ([Rosenfeld et al., 2020](#)). Both species are capable of reducing selenate and selenite to  $\text{Se}^0$  or even to  $\text{Se}^{2-}$  volatiles under aerobic conditions alongside the simultaneous oxidation of manganese ( $\text{Mn}^{\text{II}}$ ). Rosenfeld and co-workers had previously compared *P. sporulosum* and *Stagonospora* sp. to four other ascomycetes (*Acremonium strictum*, *Alternaria alternata*, *Plectosphaerella cucumerina* and *Pyrenochaeta* sp.) isolated from sites contaminated with heavy metals ([Rosenfeld et al., 2017](#)). All species showed a high tolerance of Se oxyanions and in most cases the ability to reduce selenite and selenate to  $\text{Se}^0$ , the exception being *P. sporulosum* which lacks the ability to reduce selenate. On the other hand, the ability of the yeast-like dematiaceous fungus *Aureobasidium pullulans* to reduce selenite has recently been confirmed, along with the zygomycete *Mortierella humilis* and the ascomycetes *Trichoderma harzianum* and *Phoma glomerata* ([Liang et al., 2019](#)). Furthermore, *P. glomerata* was recently shown to precipitate intracellular and extracellular  $\text{Se}^0$  nanoparticles when grown on medium supplemented with selenite ([Liang et al., 2020](#)). *Aspergillus* strains are also known to reduce selenite ([Kimura et al., 2014](#)) or selenate ([Urik et al., 2016](#)) and produce  $\text{Se}^0$  nanoparticles by the reduction of selenite, which accumulate mainly on the surface of the mycelial cell walls ([Li et al., 2018](#)). Recently, pellets of the fungus *Aspergillus niger* KP were used in an airlift reactor to remove selenite from wastewater, demonstrating the potential of this species for environmental remediation ([Negi et al., 2020](#)). Interestingly, field-emission transmission electron microscopy (FE-TEM) images revealed that  $\text{Se}^0$  nanoparticles formed within the fungal cells, suggesting the intracellular conversion of selenite into these  $\text{Se}^0$  nanostructures.

#### 3.5.4.2 Basidiomycetes

Red coloring, indicating the accumulation of amorphous  $\text{Se}^0$ , has been reported in cultures of the basidiomycetes *Lentinula* (obsolete name, *Lentinus*) *edodes*, *Pleurotus ostreatus*, *Ganoderma lucidum* and *Grifila frondosa*, which form  $\text{Se}^0$  nanoparticles when grown in media containing selenite ([Vetchinkina et al.,](#)

2016). In particular, *L. edodes* and *G. frondosa* accumulated  $\text{Se}^0$  nanoparticles predominantly within the mycelia and – to a limited extent – on the hyphal surface. In contrast, *G. lucidum* and *P. ostreatus* formed  $\text{Se}^0$  nanoparticles mostly in the growth medium. *L. edodes* reduced 0.3 mM selenite added to the growth medium, producing intracellular electron-dense spherical formations of  $\text{Se}^0$ , but cannot reduce 0.3 mM selenate under the same conditions (Vetchinkina *et al.*, 2013). Furthermore, the edible button mushroom *Agaricus bisporus* and the horse mushroom *Agaricus arvensis* reduced selenite and formed spherical  $\text{Se}^0$  particles with diameters of 100–250 and 150–550 nm, respectively (Vetchinkina *et al.*, 2019). The widely cultivated basidiomycete mushroom *Flammulina velutipes* can reduce selenite to  $\text{Se}^0$  when grown on a substrate containing optimal selenite concentrations of 0.03–0.1 mM to enrich the fungal biomass with dietary Se. In these conditions the strain is also able to form  $\text{Se}^0$  nanoparticles, possibly as a detoxification mechanism (Wang *et al.*, 2016).

The ligninolytic basidiomycete *Phanerochaete chrysosporium* can also reduce selenite and selenate, but only forms  $\text{Se}^0$  from selenite (Espinosa-Ortiz *et al.*, 2015a, b). Although the detailed mechanisms of selenate and selenite reduction are unknown, this species is likely to use a GSH-dependent mechanism for at least the reduction of selenite as proposed for bacteria (Debieux *et al.*, 2011; Xu *et al.*, 2014). Transmission electron microscopy (TEM) revealed that most Se nanoparticles were compartmentalized in the fungal cell but not equally distributed throughout the hyphae (Espinosa-Ortiz *et al.*, 2015a). *P. chrysosporium* has been tested as a means to bioreduce selenite from wastewater and recover non-toxic elemental Se in the form of  $\text{Se}^0$  nanoparticles (Espinosa-Ortiz, 2016). There is evidence for synergy between *P. chrysosporium* and the bacterium *Delftia lacustris* in reducing selenite to  $\text{Se}^0$  with contextual phenol degradation, offering another promising biotechnological approach for the bioremediation of polluted environmental matrices (Chakraborty *et al.*, 2019).

### 3.5.5 Selenium reduction by cell extracts

The synthesis of Se nanorods or nanoparticles was demonstrated in fungal extracts. In the plant pathogen *Alternaria alternata*, an ascomycete,  $\text{Se}^0$  nanostructures synthesis was induced by adding 1 mM sodium selenate to the growth medium after removing the mycelium (Sarkar *et al.*, 2011, 2012). Extracellular and intracellular extracts, even from cultures of different basidiomycetes, were shown to generate  $\text{Se}^0$  nanoparticles following sodium selenite addition (Vetchinkina *et al.*, 2018).

To conclude, focusing on the actual involvement of fungi in the biotransformation reactions of selenium compounds, with particular reference to the oxyanions selenite and selenate, assumes a prominent ecological and practical relevance where it is considered that fungal species isolated from the rhizosphere of selenium hyperaccumulator plants represent a significant fraction of the

microbiome that interacts with the root systems of these botanical species (Wangeline *et al.*, 2011).

### 3.6 FUTURE PERSPECTIVES

We are now beginning to understand the mechanisms underlying the potential active transport of biogenic nanostructured  $\text{Se}^0$  particles from the intracytoplasmic compartment to the outside environment, that not only provides new fundamental knowledge about microbial transport pathways and adaptations to stress, but could also be exploited for bioremediation of contaminated sites, mineral recovery in nanostructured forms and nutrient biofortification of food crops and feedstuff for livestock. The growing evidence for important interactions between oxidized species of selenium and diverse members of *Archaea* is of great impact in biotechnology. This topic is still in its infancy, but early results provide evidence that Se plays a key role in the biogeochemistry of thermo-acidophilic and halophilic extreme environments, and also suggest that methylophilic methanogens could be used in anaerobic bioreactors intended for the treatment of different Se-laden waste streams to recover nanostructured elemental  $\text{Se}^0$  following the transformation of Se oxyanions.

The burgeoning exploration of the relationship between diverse fungi – yeasts, molds or mushrooms – and Se oxyanions, not only represents an important theoretical advance but could also lead to the exploitation of fungi for the bioremediation of Se-contaminated environments or the biofortification of edible fungi with dietary Se. Microbes in the rhizosphere and inner tissues of plants can also facilitate the reduction of selenate or selenite in the soil, suggesting these microbes could be utilized for both phytoremediation (Lampis *et al.*, 2009; Staicu *et al.*, 2015) and the biofortification of crops (Acuña *et al.*, 2013). The endophytic bacterium *Pseudomonas moraviensis* ssp. *stanleyae*, isolated from the roots of the hyperaccumulator *Stanleya pinnata* growing on seleniferous soil in the Pine Ridge Natural Area (Colorado, USA), was shown to tolerate 150 mM selenate and 120 mM selenite, and to reduce selenite efficiently under aerobic and anaerobic conditions, producing extracellular  $\text{Se}^0$  nanostructures (Staicu *et al.*, 2015). Moreover, *Bacillus mycoides* SeITE01 and *S. maltophilia* SeITE02 were shown to enhance the phytoextraction efficiency of *Brassica juncea* plants exposed to selenite or selenate in bio-augmented water-filtering artificial beds (Lampis *et al.*, 2009).

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