Microbial reduction of selenium oxyanions by *Anaeromyxobacter dehalogenans*  

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1. Introduction  

Selenium is of significance in biology as a key trace element required by most living organisms (Madigan et al., 2000). Despite its importance in cellular functions, high concentrations of selenium, particularly in the forms of selenate (SeO\(_4^{2-}\)) and selenite (SeO\(_2^{3-}\)), are acutely toxic (Hamilton, 2004; Wu, 2004), prompting a need to study the biochemistry of selenium and to develop strategies for the effective control of selenium in the environment (Gadd, 1993; Lovley, 1995). The occurrence of toxic levels of selenium in the environment could be attributed to both natural and anthropogenic sources, as high levels of selenium have been reported in naturally seleniferous regions (Hamilton and Buhl, 2005; Lemly, 1985; Presser et al., 1994) and areas impacted by the mining and smelting industries (Banuelos and Ajwa, 1999; Nriagu and Wong, 1983; Wayland et al., 2006). The chemical and geological distribution of selenium is largely influenced by the natural biogeochemical cycling of selenium, where microbial activities play crucial roles (Oremland, 1994). It is known that the highly toxic soluble selenium oxyanions (SeO\(_4^{2-}\) and SeO\(_2^{3-}\)) can be converted to the much less toxic insoluble elemental selenium, either as a form of microbial dissimilatory respiration or detoxification mechanism (Stolz and Oremland, 1999). Thus, studying the interactions between selenium and microorganisms is of primary importance in understanding the mode of toxicity and fate of selenium.

Such interactions are particularly relevant with regard to *Anaeromyxobacter dehalogenans* strains, a group of energetically versatile δ-Proteobacteria best known for their potentials in the bioremediation of contaminant mixtures of heavy metals and radionuclides via anaerobic respiration (He and Sanford, 2003; North et al., 2004; Petrie et al., 2003; Wu et al., 2006). The significance of *A. dehalogenans* in the global cycling of redox active elements is further supported by its ecological importance demonstrated by the detection of these bacteria in diverse habitats around the world (Hori et al., 2007; Ikenaga et al., 2003; Petrie et al., 2003; Sanford et al., 2002; Treude et al., 2003). For this reason, we investigated the ability of *A. dehalogenans* to biotransform toxic selenium oxyanions, with applications to bioremediation in mind.

2. Methods  

2.1. Bacterial strains and culture conditions  

*A. dehalogenans* strains 2CP-1 (ATCC BAA-258) and 2CP-C (ATCC BAA-259) were routinely grown in 160-ml glass serum bottle with 100 ml of degassed mineral salts medium or in 30-ml anaerobic...
culture tubes with 10 ml medium and closed with butyl rubber stoppers and aluminum seals. The mineral salts medium used for all cultivation was prepared and handled as previously described (He and Sanford, 2002). Cultures were incubated in the dark at 30 °C under anaerobic conditions with acetate (10 mM) as the electron donor and fumarate (5 mM) as the electron acceptor. Fresh cultures were initiated by transfers of 1% (vol/vol) inoculum to fresh medium from grown cultures after fumarate was depleted and cell growth had stopped. Strict anaerobic techniques were used throughout all of the experimental manipulations as previously described (He et al., 2009).

2.2. Reduction of selenium oxyanions by A. dehalogenans

Cell suspensions of A. dehalogenans were prepared by harvesting late-log-phase cultures by centrifugation, washing, and re-suspending in fresh mineral salts medium to the desired optical density for testing. Various concentrations of sodium selenite or sodium selenate were added to triplicate cultures of late-log-phase cells; killed controls where selenium oxyanions were added to blank medium without A. dehalogenans cells; and cell-free controls where selenite or selenate was added to the supernatants of late-log-phase A. dehalogenans cultures obtained by filtering through a 0.22 μm membrane filter. These controls were set up when needed to assess the possibilities of non-specific chemical reduction unrelated to cellular activities.

2.3. Analytical methods

Cell density of cultures was monitored spectrophotometrically as optical density at 600 nm wavelength (OD₆₀₀). Aqueous Se was quantified with atomic absorption spectroscopy (AAS) using a model 210VGP atomic absorption spectrophotometer (Buck Scientific, East Norwalk, Connecticut, USA). Sample preparation and AAS procedure followed a previously described protocol (Kargbo and Chatterjee, 2005) with the addition of 2% (w/v) nickel nitrate solution to further determine the oxidation state of the selenium identified (Ravichandran et al., 2000). In controls where active cells were excluded or inactivated, demonstrating the enzymatic nature of the Se(IV)-reducing activity.

On the other hand, similar experiments did not indicate the reduction of Se(VI), in the form of Na₂SeO₄, by strain 2CP-1, because neither a decrease in aqueous Se concentration nor the production of a red precipitate was observed (Fig. 1).

3. Results and discussion

3.1. Biotransformation of Se oxyanions by A. dehalogenans

The ability of A. dehalogenans to reduce Se(IV) (Na₂SeO₃) was first tested on A. dehalogenans strain 2CP-1 cell suspensions (Fig. 1). Se(IV) concentrations decreased promptly following the addition of 450 μM Se(IV) to the cell suspensions, indicative of Se(IV) reduction. A reddish precipitate characteristic of elemental selenium (Se(0)) was formed simultaneously with the decrease in Se(IV) concentration, showing that selenium was transformed to amorphous elemental selenium (Tomei et al., 1992). In comparison, no significant changes in Se(IV) concentration were observed in controls where active cells were excluded or inactivated, demonstrating the enzymatic nature of the Se(IV)-reducing activity.

3.2. Elemental analysis of selenium precipitates

The product of the biotransformation of Se(IV) by strain 2CP-1 was a dense reddish precipitate. To identify the composition of this product, elemental analysis was performed using environmental scanning electron microscopy (ESEM) and energy-dispersive X-ray spectrometry (EDXS) analysis (Fig. 2). In the EDXS spectrum, a dominant energy peak with 1334 counts was detected at 1.4 keV, which is characteristic of Se (Dungan et al., 2003), indicating that Se was the major constituent of this precipitate (Fig. 2A).

X-ray photoelectron spectroscopy (XPS) analysis was conducted to further determine the oxidation state of the selenium identified by EDXS in the red precipitate produced in Se(IV) reduction by strain 2CP-1, which found a characteristic peak for Se(0) at 54.5 eV but not Se(VI) at 59 eV (Fig. 2B), confirming the presence of elemental selenium in the precipitate as the product of Se(IV) reduction by strain 2CP-1 (Meng et al., 2002).

![Fig. 1. Se(IV) reduction by A. dehalogenans strain 2CP-1 cultures (OD₆₀₀ = 0.09). ∙: cell-free controls; ●: and killed controls; ☐: Sodium selenite was added to strain 2CP-1 cultures grown with fumarate as the electron acceptor and excess acetate as the electron donor. The inset shows the changes in Se(VI) concentration when sodium selenite was added to replicate cultures of strain 2CP-1. Results are the averages of triplicates cultures with error bars indicating the standard deviations.](image-url)
Inhibitory effect of selenite on Se(IV) reduction

It has been observed in previous studies that Se(IV) reduction could be inhibited at higher Se(IV) concentrations (Ike et al., 2000), which could limit the potential application of this advantageous microbial process in Se detoxification. To test the presence of this potential inhibitory effect in strain 2CP-1 cultures, varying concentrations of Na2SeO3 was added to strain 2CP-1 cultures (OD600/C25 = 0.09) and subsequent changes in selenite concentration were monitored. Indeed, selenite at concentrations above 900 μM resulted in significant inhibition of the Se(IV) reduction activity since greater than 40% and 70% of the 900 μM and 1200 μM selenite added, respectively, could not be reduced by strain 2CP-1, following 45 h of incubation (Fig. 3A). In contrast, at a lower concentration (450 μM selenite), nearly 100% of the Se(IV) was reduced in just 9 h, indicating that higher levels of selenite had an inhibitory effect on Se(IV)-reducing activity by strain 2CP-1.

The inhibitory effect of higher concentrations of selenite on Se(IV) reduction was further illustrated in Fig. 3B, where two sets of triplicate cultures of strain 2CP-C received the same amount of Se(IV), i.e. 900 μM of Na2SeO3; however, the 900 μM selenite was added in two different concentration schemes: either as a single spike of 900 μM to one set of cultures or as two consecutive spikes of 450 μM to the other set, so that the two sets of triplicate cultures differed only in the concentration but not the total amount of Se(IV) received. The addition of 900 μM selenite in two 450 μM spikes, i.e. lower Se(IV) concentration, resulted in the reduction of approximately 89% of the 900 μM Se(IV) added in 34 h. In contrast, only 54% of the 900 μM Se(IV) was reduced by strain 2CP-1 during the same period of time (34 h) when selenite was added as a single spike, i.e. higher Se(IV) concentration, further evidence of the inhibitory effect of Se(IV) at higher concentrations. However, the inhibition of Se(IV) reduction by A. dehalogenans appears to occur at Se(IV) concentrations slightly lower than those in other Se(IV)-reducing bacteria, where the inhibitory concentration of Se(IV) is typically greater than 1 mM (Hunter and Manter, 2009).

It should also be noted that the Se(IV)-reducing activity declined progressively as evidenced by the change in initial Se(IV) reduction rate in the cultures that received two consecutive spikes of selenite, where the initial Se(IV) reduction rate decreased from 99 μM/h in the 1st spike to 33 μM/h in the 2nd spike (Fig. 3B). These results indicate that the reduction of selenite by strain 2CP-1 was accompanied by the gradual loss of Se(IV)-reducing activity. This loss of Se(IV)-reducing activity, however, was not a result of cell aging, since the Se(IV)-reduction activity remained unchanged in strain 2CP-1 cultures resting for up to 48 h (data not shown). It is possible that a negative impact on cellular activity might have occurred even at selenite concentrations below 450 μM, when no apparent inhibition was observed (Fig. 3A).

Nevertheless, the ability of strain 2CP-1 to readily convert Se(IV) to less toxic Se(0) at concentrations up to 450 μM suggests potential applications in bioremediation of toxic Se accumulated in certain environments, where the concentrations of Se typically reach micromolar levels which are much lower than the concentrations causing significant inhibitory effects as observed in this study (Engberg and Sylvester, 1993; Stolz and Oremland, 1999).

Fig. 2. Characterization of Se(IV) reduction product by EDXS and XPS analysis. (A) A representative EDXS spectrum of the red precipitate found in A. dehalogenans strain 2CP-1 cultures containing Na2SeO3 as the sole electron acceptor. The C and O peaks are from the sample tape used to conduct electricity. The energy range is from 0 to 5 keV. The Se peak is 1334 counts. (B) XPS analysis of the oxidation states of Se in the Se-containing red precipitate produced from microbial Se(IV) reduction by strain 2CP-1 (broken line) as well as the reference sample (solid line): a mixture of the Se-containing red precipitate and pure Na2SeO3. Characteristic peaks for Se(IV) and Se(0) are located at 59 eV and 54.5 eV, respectively.
Fig. 3. Inhibitory effect of selenite on Se(IV) reduction by A. dehalogenans strain 2CP-1. (A) Reduction of Se(IV) following single spikes of varying concentrations of Na2SeO3; and (B) Reduction of 900 µM Se(V): one single spike vs two consecutive spikes, with the arrow indicating the 2nd spike. Se(IV) reduction was consistent with that of strain 2CP-1 (Fig. 3B), as indicated in this study (Figs. 1 and 4) suggests that the Se(IV)-reducing activity by A. dehalogenans strains have been recently recognized as a group of versatile bacteria ubiquitously distributed in diverse natural environments best known for their bioremediation capacity to immobilize heavy metals and radionuclides, the discovery reported in this communication adds another dimension to the potential environmental applications of these organisms. It is also shown that the Se(IV)-reducing activity by A. dehalogenans could be significantly inhibited by high levels of Se(IV). With Se(IV) reduction being proposed as a detoxification mechanism in other microorganisms, it is likely that the inhibition of Se(IV) reduction by Se(VI) was a result of the severe toxicity of Se(IV) at elevated concentrations. Further studies are needed to elucidate the mechanisms of Se(IV) reduction and its inhibition by Se(VI) in order to understand the potential roles of these ecologically important microorganisms in the biogeochemical cycling of selenium and the remediation of toxic forms of selenium.

3.4. Se(IV)-reducing activity in A. dehalogenans strain 2CP-C

To test the ability of other A. dehalogenans strains to reduce Se(IV), changes in Se(IV) concentration were monitored in the cultures of another Anaeromyxobacter strain 2CP-C (OD600 ~ 0.09), which is also capable of utilizing a series of alternative electron acceptors (Sanford et al., 2002). Clearly, strain 2CP-C was capable of Se(IV) reduction as strain 2CP-1 (Fig. 4).

The Se(IV)-reducing activity of strains 2CP-1 and 2CP-C demonstrated in this study (Figs. 1 and 4) suggests that the Se(IV)-reducing capacity may be widely distributed among A. dehalogenans strains. The ability to detoxify selenium via Se(IV) reduction also extends the bioremediation potential known for these organisms, including halogenated phenols, heavy metals, and radionuclides (He and Sanford, 2003; Sanford et al., 2002, 2007).

Notably, strain 2CP-C was more tolerant to selenium than strain 2CP-1 with the complete reduction of 900 µM of SeO2⁻, which was not the case for strain 2CP-1 (Fig. 4). However, the progressive decline of Se(IV)-reducing activity during the very process of Se(IV) reduction was consistent with that of strain 2CP-1 (Fig. 3B), as the initial Se(IV) reduction rate of 119 µM/h following the 1st spike of 450 µM selenium declined to 55 µM/h during the reduction of the 2nd spike of 450 µM selenium (Fig. 4).

The limited scope of this study made it infeasible to ascertain the mechanism of this inhibitory effect. However, the reduction of Se oxyanions to insoluble elemental selenium has been suggested as a detoxification mechanism in a number of microorganisms (Garbuio et al., 1995; Lortie et al., 1992; Losi and Frankenberger, 1997; Tomei et al., 1995). Thus, the reduction of Se(IV) by A. dehalogenans might be a mechanism of detoxification as well. Since the Se(IV) concentrations tested in this study were considerably higher than those typically encountered in natural environments (Engberg and Sylvester, 1993; Stolz and Oremland, 1999), cellular activity could be negatively impacted despite the occurrence of Se(IV) reduction. On the other hand, the potential inhibition of Se(IV)-reducing activity by Se(IV) might not be of practical concern because of the relatively low concentration of Se(IV) in the environment.

4. Conclusions

In this study, we demonstrated the ability of two A. dehalogenans strains to readily convert toxic Se(IV) to less toxic insoluble Se(0), which has the potential for the detoxification of Se(IV) in the environment. Since A. dehalogenans strains have been recently recognized as a group of versatile bacteria ubiquitously distributed in diverse natural environments best known for their bioremediation capacity to immobilize heavy metals and radionuclides, the discovery reported in this communication adds another dimension to the potential environmental applications of these organisms. It is also shown that the Se(IV)-reducing activity by A. dehalogenan could be significantly inhibited by high levels of Se(IV). With Se(IV) reduction being proposed as a detoxification mechanism in other microorganisms, it is likely that the inhibition of Se(IV) reduction by Se(VI) was a result of the severe toxicity of Se(IV) at elevated concentrations. Further studies are needed to elucidate the mechanisms of Se(IV) reduction and its inhibition by Se(VI) in order to understand the potential roles of these ecologically important microorganisms in the biogeochemical cycling of selenium and the remediation of toxic forms of selenium.

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References


