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Selenite detoxification by *Bacillus* spp isolated from indigenous polluted sites

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Abstract

This investigation was proposed to monitor the ability of isolated *Bacillus* spp. to transform toxic forms of selenium (selenium oxyanions) to non toxic selenium. These strains reduced up to 89% selenite on average at 37℃ after time of incubation. At higher initial concentrations (100, 200, 400, 600 and 800 µg ml^{-1}), reduction value dropped to 31%. In the presence of other metals stresses (Co, Hg and Cr at a concentration of), the average selenite reduction percentage was 48%. This reduction value shifts from 87% to 94% with the increase in incubation time (from hrs to hrs). Reduction potential of these strains decreased 81% to 27% at various initial selenium concentrations in N-broth and acetate minimal media, respectively. With the increase in the sodium concentration of the media, the measured selenite reduction was above 95%. After exposure to the UV treatment *B. pichinoty* lost its ability to reduce selenite while *B. endophyticus* and *B. foraminis* reduced up to 96% and 71%, of selenite, respectively.

Keywords: Selenite, bacteria, *Bacillus*, Bioremediation, heavy metals

1. Introduction

The main threats to human beings from heavy metals in their toxic forms are associated with exposure to their toxic insoluble oxyanions (Lampis *et al*., 2014). For proper growth and development these ions required in little amount as they are the part of vital enzymes, proteins and are involved in various physiological processes (Wang *et al*., 2014). Some elements are although indispensable for living system at low concentration but at higher concentration they become toxic and deleterious for growth and life (Yao *et al*., 2003). Bacteria living under subsurface are able to detoxify and degrade metals and other pollutants by the process of situ bioremediation (Chapelle, 2001). The bacterial removal of toxic metals in these days is very important and considered a vital process for better environment (Jonathan, 2003). Micro-organisms also have resistance mechanisms which introduce convert metal oxidation states (Tetteh *et al*., 2014), organometals, and radionuclide contaminants (Bajaj *et al*., 2014). Bacterial reduced the mobility of contaminats by reducing it in less harm state (Adeniji, 2004). Many microorganisms like bacteria, protozoan's and algae are utilized for metal removal (Pena-Castro, 2004; Munoz *et al*., 2006; Munoz, 2006). Selenium is essential in a way that it is very crucial for organisms but in very low quantity and if this disturb then it is toxic (Zheng *et al*., 2014, Kim *et al*., 2014). Selenium is required for both animals and plants for their proper growth (Li *et al*., 2014) while it may be toxic when present in high amount (Tinggi, 2003, Zheng *et al*., 2014). As part of 21st amino acid - selenocysteine - that is present in many enzymes is essential to all living organisms (Gouget *et al*., 2005). Selenium comes in environment through fertilizers, fossils fuel, metal processing and many others activities (Broadley *et al.*, 2006). Bacteria isolated from selenite rich environment have developed a variety of resistance mechanisms, for instance; the oxidation, reduction or methylation of inorganic and organic selenium species (Ayano *et al*., 2014) some bacteria can use the selenate as electron acceptor (Gouget *et al*., 2005). Various environmental factors like time duration, selenium concentration, pH, temperature and moisture may impact on these reactions (Lenz, 2008). The gene fnr is involved for the reduction of selenite to elemental selenium (Yee *et al*., 2007). This study aims to determine the bio-transformation ability of *Bacillus* spp. at different physico-chemical factors so that they may be used practically for bioremediation purposes.

2. Material and Methods

Three isolated and identified strains (*Bacillus foraminis*-YAK-1 Accession No. JX203248, *Bacillus endophyticus*-YAK-7 Accession No. JX203252, *Bacillus pichinotyi*-YAM-2 Accession No. JX203257) were taken and routinely in N-Agar amended with 500 µg ml^{-1} of sodium selenite (Na_2SeO_3).

2.1 Selenite reduction assay

Reduction experiments were undertaken in aerobic conditions. Ten ml of N-broth amended with Na_2SeO_3 in 20ml test tubes stopped with a cotton plug were prepared and autoclaved. Medium was inoculated with 24 hours old culture and incubated at 37 ℃ for 48 hours at two initial selenite (200 and 400 µg ml^{-1}) concentration. After incubation selenite content was estimated using a modified method of Keka *et al.* (2011).

2.2 Selenite content determination

With little modification of Keka *et al.* (2011) method was used for the estimation of selenium content. After the glass test tubes containing 10 ml of N-broth were autoclaved, aliquots of sterile 1M Na_2SeO_3 solution was taken and after inoculation were incubated for 48 hours at 37 ℃. Red colored culture broth was stirred others centrifuge tubes. Solution was centrifuged (5000 rpm) for ten minutes; pellet and supernatant were separated. In order to remove non-metabolized selenite, ten ml of 1 M NaCl was added to wash off the pellets. Then ten ml of distilled water poured into the centrifuge tubes and centrifuged (5000 rpm) for ten minutes. Then pellet was suspended by vortex and its absorbance was determined by spectrophotometer at 500 nm.

2.3 Effect of UV radiation on bacterial selenite reduction

Strains were exposed to UV radiation for 15, 30 and 60 minutes time duration. Colonies were selected, cultured and their ability to grow and reduce the selenite was determined.

3. Results

3.1 Bacterial strains

Three identified strains (*Bacillus foraminis*-YAK-1 Accession No. JX203248, *Bacillus endophyticus*-YAK-7 Accession No. JX203252, *Bacillus pichinotyi*-YAM-2 Accession No.JX203257) were maintained on N-agar plate supplemented with sodium selenite.

3.2 Strain characterization

These bacterial strains are gram positive and forms spores. All these strains are aerobic rods. These strains showed resistance in the presence of selenite.

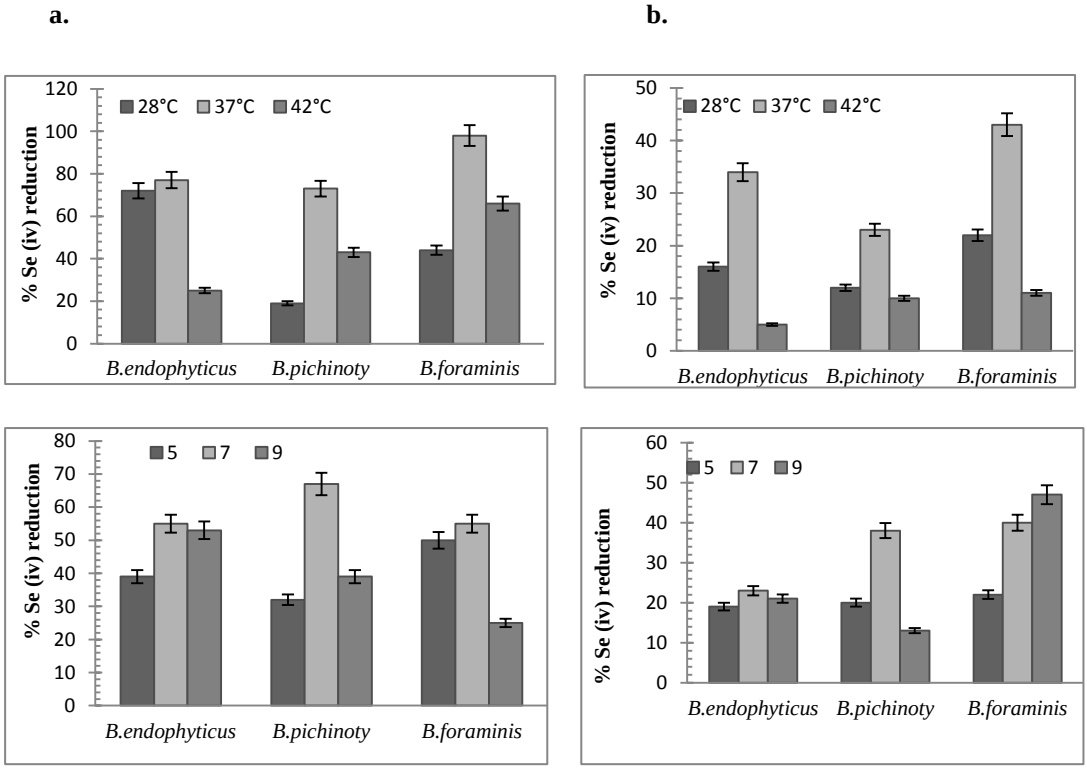

Figure 1. Effect of various temperature (28, 37 and 42 ℃) and pH (5, 7 and 9) on selenite reduction. (a) at 200 µg

ml^{-1}, (b) at 400 µg ml^{-1} of sodium selenite after 48 hours incubation period.

3.3 Selenite reduction

3.3.1 At different temperature

At 37 ℃ all of the strains had maximum reduction of selenite at both selenite concentrations i,e; 200 and 400 µg ml^{-1}. At 42 ℃ *B. foraminis* showed increased reduction potential than other strains, but marked decrease in reduction potential at higher concentration (Figure 1).

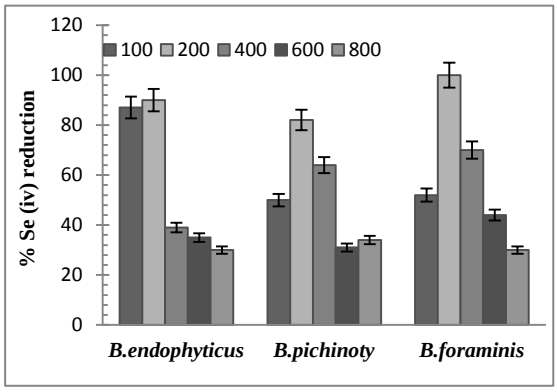

Figure 2. Effect of various initial selenite concentrations (100, 200,400, 600 and 800 µg ml^{-1}) on selenite reduction potential of bacterial strains.

3.3.2 At different initial selenite concentration

B. endophyticus and *B. pichinoty* showed the highest reduction at a concentration of 800 µg ml^{-1}. *B. foraminis* had maximum reduction potential at 400 µg ml^{-1} concentration (Figure 2).

3.3.3 At different pH

Strain *B. endophyticus* had maximum reduction potential at neutral pH when initially supplied with 200 µg ml^{-1}. *B. pichinoty* had maximum reduction at pH 7 and concentration 400 µg ml^{-1}. *B. foraminis* showed maximum reduction at pH 9 and concentration 400 µg ml^{-1} (Figure 1).

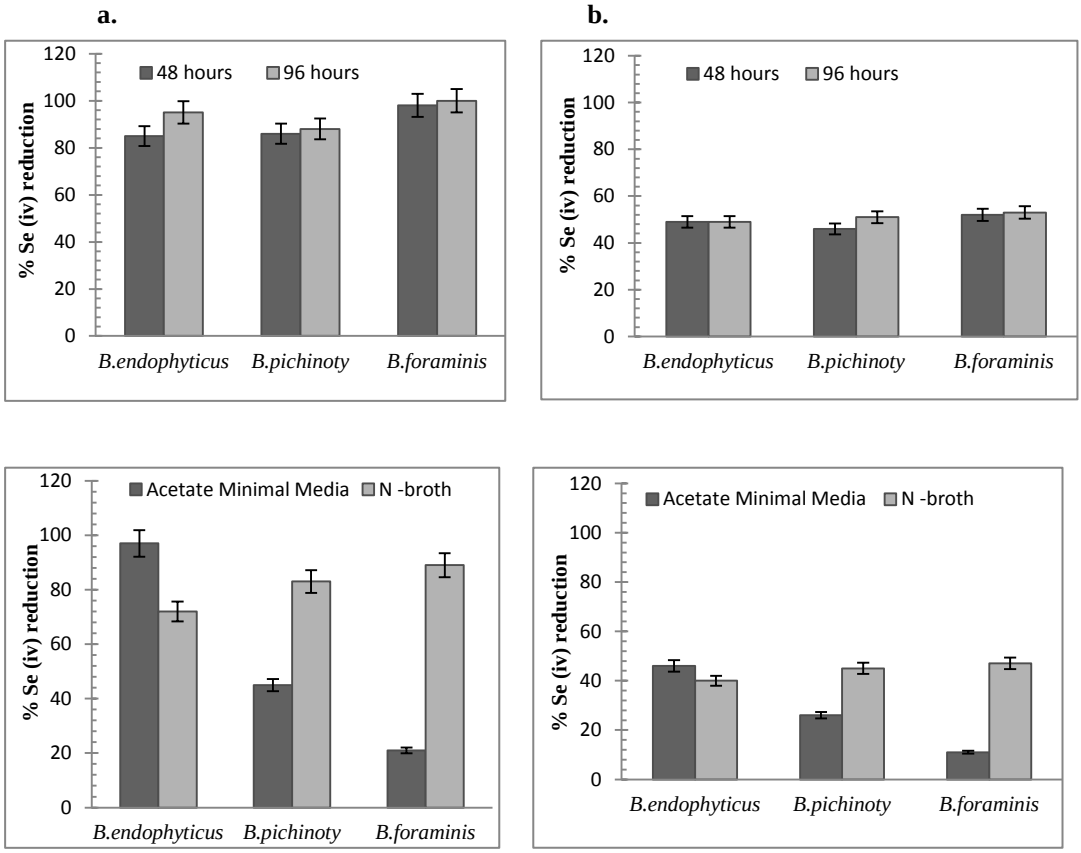

Figure 3. Effect of various incubation time (48 and 96 hrs) and media (acetate minimal media, N-broth) on

selenite reduction potential of bacterial strains (a) at 200, (b) at 400 µg ml^{-1}.

3.3.4 In presence of heavy metals

B. endophyticus and *B. foraminis* reduces selenite optimally in the presence of mercury and cobalt stress respectively, but all other strains showed decreased reduction potential in increased concentration of other heavy metals.

3.3.5 At different incubation time

A common trend was observed in all the strains that as the incubation time was increased reduction potential also increased. After 48 hours of incubation *B. foraminis* showed the highest reduction potential while *B. pichinoty* showed lowest reduction potential. After 96 hours incubation duration the same results were obtained with the slightly increase in reduction potential (Figure 3).

3.3.6 In presence of different media

B. endophytics showed maximum reduction of selenite in acetate minimal media at concentration 200 µg ml^{-1} with slightly decrease in reduction potential at higher concentration i.e. 400 µg ml^{-1}. *B. pichinoty* showed reduction less than *B. endophytics* at concentration 200 µg ml^{-1} but showed an increase in reduction at 400 µg ml^{-1}. *B. foraminis* showed minimum reduction at both concentrations with slight increase in reduction at 400 µg ml^{-1} (Figure 3).

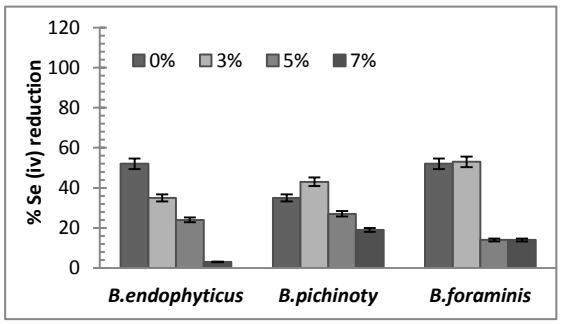

Figure 4. Effect of salinity (0%, 3%, 5% and 7%) on selenite reduction potential of bacterial strains at 400 µg ml^{-1} after 48 hours incubation period.

3.3.7 Effect of salinity on selenite reduction

At 400 µg ml^{-1} concentration of sodium selenite, *B. foraminis* showed maximum reduction in 3% salinity, *B. pichinoty* showed maximum reduction in 5% salinity and *B. endophyticus* showed maximum reduction in 7% salinity. All of these strains showed the same trend of gradual decrease in reduction potential with the increase in salinity. When the concentration of selenite is increased from 200 to 400 µg ml^{-1}, all of the strains had shown a decrease in reduction potential (Figure 4).

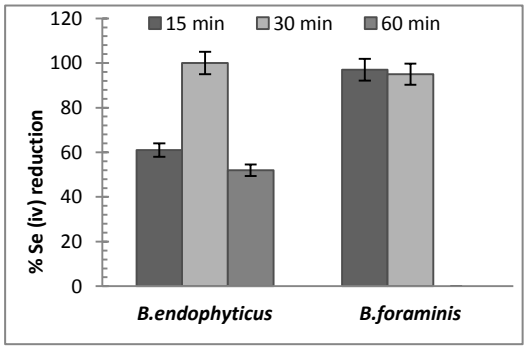

Figure 5. Effect of UV radiations on selenite reduction potential of bacterial strains (a) at 200 µg ml^{-1}, (b) at 400 µg ml^{-1} after 48 hours incubation period

3.3.8 Effect of UV treatment on selenite reduction of strains

Exposure of strains to the UV radiation for various time duration led to the loss of ability to tolerate the selenite in case of *B. pichinoty* whereas *B. endophyticus* showed optimal reduction by the culture exposed to UV radiation for 30 minutes while *B. foraminis* showed optimal reduction after 15 minutes exposure (Figure 5).

4. Discussion

Biotransformation of selenite as well as selenate into elemental selenium is known as dissimilatory selenate/selenite reduction (Narasingarao and Häggblom, 2007). A number of remediating techniques including reduction techniques have been developed for the removal of toxic forms of selenium (Dungan and Frankenberger, 1999). In present study three *Bacillus* species transformed toxic selenite into nontoxic insoluble elemental selenium aerobically at different physico-chemical parameters since its reduction under these factors prove these strains are important for bioremediation process. All of the strains exhibited optimal biotransformation at 37 ℃ and showed different optimal transformation at different pH, *B. endophyticus* reduced slenite optimally at pH range 5-7, *B. foraminis* and *B. pichinoty* at 5-9 in the presence of different concentration. With the gradual increase in the selenite content in medium all strains showed decreased reduction. When the selenite concentration reached 800 μg ml^{-1}, selenite reduction reduced to 30 to 34%. It was observed that high initial reduced reduction ability of strain as observed in *Pseudomonas stutzeri*, reduction potential decreased as selenite concentration increased above 19mM (Lortie *et al*., 1992). Since selenite reduction is not an independent process, a number of other pollutants are also present that can either inhibit or enhance the reduction process. These strains showed reduced transformation in the presence of other metals. When *Shewanella oneidensis* was grown aerobically it will reduced selenite mainly at end of stationary stage (Klonowska *et al*., 2005). These *Bacillus* spp. showed increased reduction potential over time. The amount of Se volatilized by the cultures increased with the growth of the cultures over time (Desouza *et al*., 2001). Greater the incubation time greater will be the reduction potential. The heterotrophic selenium respiring bacteria utilize carbon for selenium reduction (Oremland *et al*., 1999; Losi and Frankenberger, 1997; Cantafio *et al*., 1996). Strains exhibited improved reduction potential in acetate minimal media. All strains showed abundant growth and increased reduction in saline medium as compared to N-agar, but reduction potential was gradually decreased with increasing salinity. *B. endophyticus* and *B. foraminis* showed the ability to withstand the prolonged UV rays exposure while retaining their ability to bio-transform the selenite while *B. pichinoty* was sensitive to UV rays and showed no growth at all. These strains showed their remarkable ability to transform and detoxify toxic oxy-anion of selenium present in industrial effluents over the wide environmental parameter range.

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