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Bacillus sp. strain QW90, a bacterial strain with a high potential application in bioremediation of selenite

Mohaddeseh Khalilian[1], Mohammad Reza Zolfaghari[2*], Mohammad Soleimani[2], Mohammad Reza Zand Monfared[2]

[1]MSc of Microbiology, Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Qom Branch, Qom, Iran
[2]Assistant Professor, Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Qom Branch, Qom, Iran
[3]MSc of chemistry, Department of Chemistry, Faculty of Basic Sciences, Islamic Azad University, Qom Branch, Qom, Iran

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***Corresponding author:** Mohammad Reza Zolfaghari, Islamic Azad University, 15 khordad Street, Qom, Iran. Tel: +98 9124513783,
Email: Mreza.zolfaghary@gmail.com

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Abstract

Introduction: Selenium oxyanions are toxic to living organisms at excessive levels. Selenite can interfere with cellular respiration, damage cellular antioxidant defenses, inactivate proteins by replacing sulfur, and block DNA repair. Microorganisms that are exposed to pollutants in the environment have a remarkable ability to fight the metal stress by various mechanisms. These metal-microbe interactions have already found an important role in bioremediation. The objective of this study was to isolate and characterize a bacterial strain with a high potential in selenite bioremediation.
Methods: In this study, 263 strains were isolated from wastewater samples collected from selenium-contaminated sites in Qom, Iran using the enrichment culture technique and direct plating on agar. One bacterial strain designated QW90, identified as *Bacillus* sp. by morphological, biochemical and 16S rRNA gene sequencing, was studied for its ability to tolerate high levels of toxic selenite ions by challenging the microbe with different concentrations of sodium selenite (100-600 mM).
Results: Strain QW90 showed maximum Minimum Inhibitory Concentration (MIC) to selenite (550 mM) and the maximum selenite removal was exhibited at 30 degrees C, while the activity was reduced by 20% and 33.8% at 25 and 40 degrees C, respectively. The optimum pH and shaking incubator for the removal activity were shown to be 7.0 and 150 rpm at 50.7% and 50.8%, respectively. Also, the concentration of toxic sodium selenite (800 μg/ml) in the supernatant of the bacterial culture medium decreased by 100% after 2 days, and the color of the medium changed to red due to the formation of less toxic elemental selenium.
Conclusion: This study showed that the utilization of enrichment culture technique in comparison to the direct plating on agar leads to better isolation of selenite resistant bacteria. Bacterial strain was resistant to high concentrations of selenite and also it reduced selenite to red elemental selenium. Therefore, this microorganism could be further used for bioremediation of contaminated sites.
Keywords: Bioremediation, Bacterial strain, MIC, Selenite

Introduction

Selenium was first recognized in 1817 by J.J. Berzelius and is the 66th most abundant element in the earth's crust at only 50 ppb (1). It is obtained in limited ways, such as a by-product of the electric smelting of copper, and is an important material used in photoelectric devices, photosensitive drums used in dry copying, semiconductors, and the colorization and decolorization of glasses. Biologically, selenium is an essential element used for the synthesis of selenocysteine contained in selenoproteins, such as mammalian glutathione peroxidase and bacterial formate dehydrogenase. However, exposure to higher concentrations of selenium is toxic (2). High intake of selenium in humans can lead to respiratory distress, liver and kidney necrosis and cell death. Chemical detoxification of polluted sites can be expensive and often results in secondary effects in the environment (3). Therefore, it is important to understand how environmental selenium is controlled. Selenium has several oxidation states in the environment, i.e. selenate (+VI), selenite (+IV), elemental selenium (0), selenide (-II), and organic selenium (-II). It is also known that prokaryotes play a pivotal role in its oxidation and reduction (2).
Se-reducing bacteria is ubiquitous and occurs in diverse terrestrial and aquatic environments. A few microorganisms have been well characterized for their ability to re-

duce toxic selenite ions into non-toxic elemental forms under aerobic and anaerobic conditions (4).

The optimization of biological remediation processes depends on an understanding of the biology involved, and if bacterial inoculation is needed, the identification and characterization of microorganisms can best carry out the desired remediation. The present study was an attempt to isolate and characterize the microorganism capable of transforming toxic SeO_3^{2-} into non-toxic elemental selenium and to investigate its ability in selenite removal from contaminated sites.

Methods

Chemicals

Sodium selenite anhydrous was obtained from Applichem (Germany) and sodium sulfide and thionine dye were acquired from Merck (Darmstadt, Germany). The stock solutions were prepared in distilled water and maintained at 4 °C following sterilization by microbiological filter (0.22 μm). Working solutions were stored at 4 °C for up to 5 days.

Sample collection and isolation of metalloid-tolerant bacterial strains

Selenium-contaminated water samples were collected from the industrial area of Qom in Iran. Totally, 263 bacterial strains were isolated using the enrichment culture technique and direct plating on agar at 34 °C in a shaking incubator (150 rpm) and pH 7.0 for 48 h in LB broth (Luria Bertani broth) supplemented with 10 mM selenite under aerobic conditions. Red colonies, indicating the reduction of selenite, were re-streaked on LB agar without selenite to confirm that the color was not due to pigmentation. The pure cultures were isolated and maintained on selenite supplemented plates. Filter sterile 10 mM sodium salts of selenite were added to the LB medium after autoclaving (5). Among the strains isolated, the strain named QW90 showed the highest tolerance toward this sodium selenite and was selected as a model strain for further experiments.

Determination of Minimum Inhibitory Concentration (MIC)

In order to determine MICs, the strains were grown in LB agar medium supplemented with sodium selenite at increasing concentrations (100-600 mM) and were incubated at 34 °C, for 72 h. Each plate was prepared in triplicates (6).

Characterizations of the bacterial isolates

Morphological characterizations, such as colony and cell morphology, Gram-reaction, motility etc., were performed as described by Ghosh *et al* (7). Physiological and biochemical tests were carried out according to standard protocols described by Gerhardt *et al* and Ventosa (8,9).

To determine the optimum temperature and pH for the growth of the strain, the cultures were incubated at a temperature range of 15-50 °C with intervals of 5 °C and pH values of 5-10.5, pH values below and above 6 were adjusted by sodium acetate and Tris-HCl buffer, respectively. Also, growth of the strain was evaluated at different percentage of NaCl values (0-30 % NaCl) (10).

Selenite removal experiments

Cells were cultured in 100 ml Erlenmeyer flasks containing 25 ml of LB broth supplemented with 800 μg/ml sodium selenite. The basal medium was inoculated with 1% of 1.5 × 108 cfu/ml of the bacterial suspensions and incubated aerobically at 30 °C and the pH value of 7 on a shaking incubator (150 rpm) for 2 days. The cells were centrifuged at 10000 rpm for 10 minutes and the supernatants were used to determine the residual sodium selenite through slightly modified kinetic spectrophotometric method based on the catalytic role of selenite in reducing thionine dye by sulfide ions (11).

Factors affecting selenite removal

The capacity of selenite removal by the strain was evaluated at different pH values (5-10.5) and temperatures (25-40 °C), and on a shaking incubator (50-200 rpm) in basal medium supplemented with 800 μg/ml sodium selenite. To evaluate the effect of initial selenium concentration, selenite removal was monitored in basal medium supplemented with varying concentration of sodium selenite (200-2000 μg/ml). All experiments were done in triplicate.

Phylogenetic analysis

Genomic DNA of the isolate was extracted with a genomic DNA extraction kit (Cinnagene) by following the manufacturer's recommended procedure. The 16S rRNA gene was amplified using the universal primers 8F (5′-AGAGTTTGATYMTGGCTCAG-3′) and 1541R (5′-AAGGAGGTGATCCAGCCGCA-3′). The amplification was done by initial denaturation at 95 °C for 5 minutes; subsequent denaturation at 95 °C for 1 minute; annealing at 66.6 °C for 1 minute; extension at 72 °C for 1 minute and final extension at 72 °C for 10 minutes. The Polymerase Chain Reaction (PCR) product was directly double-strand sequenced by Seqlab Laboratory (Germany). The analysis of DNA sequences and homology searches were completed using the BLAST algorithm for the comparison of the nucleotide query sequence against a nucleotide sequence database. Multiple sequence alignments were done using CLC Sequence Viewer version 6.5.1. Phylogenetic trees were inferred using the neighbor-joining method as implemented in the software.

Results

Strain characterizations

Among 263 strains of bacteria isolated from industrial wastewaters in Iran, one strain was selected for further study. In fact, strain QW90 showed maximum MIC to selenite (550 mM). The resistance of strain QW90 was associated with the reduction of selenite to selenium and the formation of the red elemental selenium precipitate in the medium.

According to phenotypic characterizations of the strain and in comparison to other studies, the strain was identified as *Bacillus* sp. strain QW90.

Strain QW90 was shown to be a Gram-positive, produced sporule, non-motile, strictly aerobic rod, catalase-positive, and non-oxidase. Strain QW90 could grow in a range of temperatures (25-50 °C), pH conditions (5-10.5) and percentage of NaCl range (0-30%). However, the optimum growth was seen at 30 °C, pH 7.0 and 3% NaCl. Table 1 shows some characteristics of strain QW90.

To confirm the identity of the isolate, PCR amplification and sequencing of the 16S rRNA gene were completed.

The phylogenetic tree (Figure 1) constructed by the neighbor-joining method indicated that the isolate QW90 was part of the cluster within the genus *Bacillus*. Among the described species, the closest relative of isolate QW90 was *Bacillus* sp. AB315f (FR821125).

Selenite reduction experiments

Strain QW90, which showed the maximum resistance to sodium selenite, was selected for the removal of selenite from contaminated environments. The effects of various environmental parameters in removing sodium selenite from culture medium by the strain were evaluated using sodium sulfide-thionine as an indicator. The strain also showed the reduction ability of selenite after 2 days in comparison with the control (a medium without the strain).

The decrease in the selenite concentrations during growth is shown in Figure 2. Typically, the maximum selenite removal in LB broth medium with a concentration of 800 μg/ml sodium selenite was determined to be 100% after 2 days.

As shown in Figures 3, 4 and 5, pH, temperature, and rpm had significant effects on sodium selenite removal and the maximum removal occurred at pH value of 7.0, 30 °C

Table 1. Morphological, physiological, and biochemical characteristics of strain QW90 as a selected strain.

Strain characteristic	Strain QW90	Strain characteristic	Strain QW_{90}
Cell type	Rod	**Catalase**	+
Gram Staining	+	**Oxidase**	-
Form	Irregular	**Motility**	-
Margin	Undulate	**Endol**	-
Elevation	Flat	**H_2S production**	-
Texture	Viscous	**Voges-Proskauer test**	-
Opacity	Opaque	**Methyl red test**	+
Pigmentation	White	**Citrate Simmon**	-
Spore production	+	**Diameter >5 mm**	+
Growth limit in %NaCl	0-30	**Hydrolysis**	
Growth optimum in NaCl	3	Gelatin	+
Temperature limit of growth	25-50	Starch	+
Growth optimum of Temperature	30	Casein	+
Growth limit of pH	5-10.5	**Enzyme activity**	
Growth optimum of pH	7	DNase	+
Acid production from		Urease	-
Mannitol	-	Phenylalanine deaminase	-
D-glucose	-	Lysine decarboxylase	+
Lactose	+	Nitrate reduction	+
Salicin	+	Hemolysis	+
Sucrose	-	**Using of carbon sources**	
Xylose	-	Mannitol	-
Glucose (anaerobe)	-	D-glucose	+
Mannitol (anaerobe)	-	Lactose	-
Using of nitrogen sources			
L-Methionine	-	L-Tryptophan	-
L- Arginine	-	L- Lysine	+

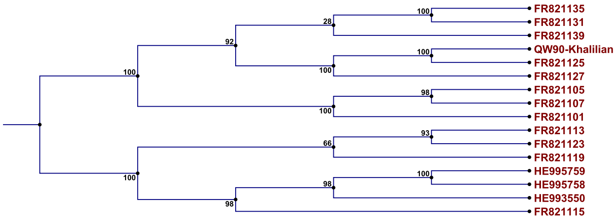

Figure 1. Neighbor-joining tree showing the phylogenetic position of *Bacillus* sp. Strain QW90 among members of rod Gram-positive bacteria.

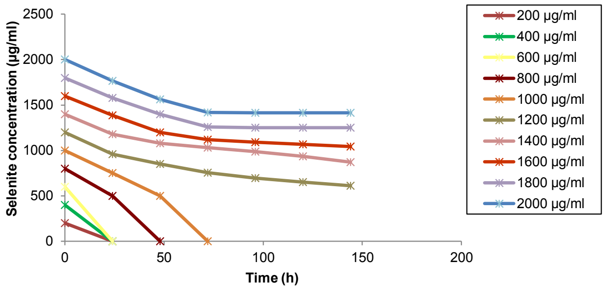

Figure 2. Different concentrations effect of selenite on their removal by strain QW90 in LB broth medium (T= 30 °C, pH=7.2 ± 0.2, rpm=150). Reduction was monitored after 24, 48, 72, 96, 120, 144 h.

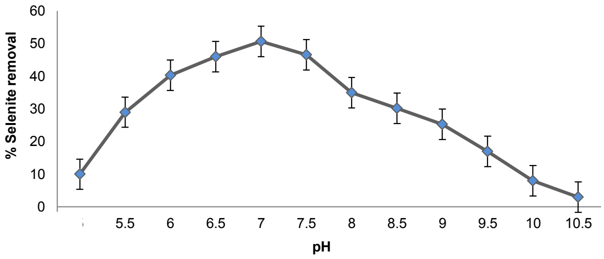

Figure 3. Effect of pH values on selenite removal by strain QW90 in LB broth medium containing 800 µg/ml selenite after 24 h (T= 30 °C, rpm=150).

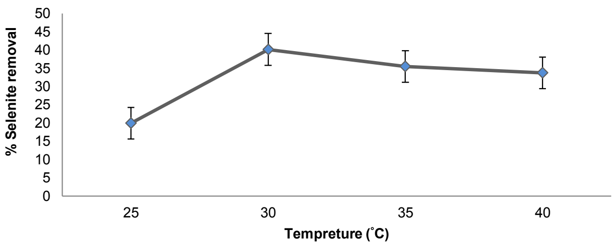

Figure 4. Temperature effect on selenite removal by strain QW90 in LB broth medium containing 800 µg/ml selenite after 24 h (pH=7, rpm =150).

and 150 rpm. At higher and lower pH, temperature and rpm, the amount of sodium selenite removal was less. The reduced removal capacities at pH value of 7, temperature of 30 °C, and shaking incubator of 150 rpm were 50.7%, 40.2% and 50.8%, respectively.

Discussion

In 2009, Kafilzadeh *et al* reported that the enrichment

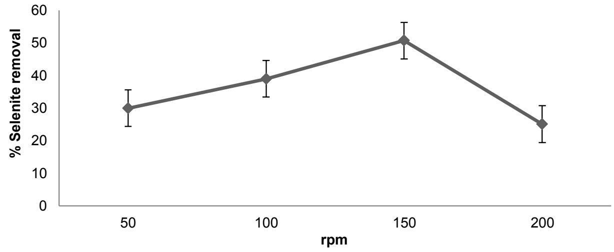

Figure 5. Shaking incubator effect on selenite removal by strain QW90 in LB broth medium containing 800 µg/ml selenite after 24 h (T= 30 °C, pH=7).

culture technique for the isolation of resistant bacteria is better. Also, the bacteria isolated in this way, show better growth in the presence of metal. Thus, the enrichment culture technique caused expression of the metal resistant genes in bacteria and its compatibility with the existing conditions (5). Also, this study showed that utilizing enrichment culture technique in comparison to the direct plating on agar leads to better isolation of selenite resistant bacteria.

We isolated 263 strains from various industrial wastewaters in Iran and evaluated their resistance patterns to sodium selenite. Strain QW90 showed maximum MIC (equal to 550 mM), which was much higher than the previous reports for *Stenotrophomonas maltophilia* SeITE02 (12), *Aeromonas salmonicida* C278 (13), *Halomonas* sp. strain MAM (10), *Rhizobium* sp. strain B_1 (14), *Tetrathiobacter kashmirensis* (15), *Bacillus fusiformis* (16), *Pseudomonas* sp. CA_5 (17) and *Bacillus cereus* strain CM100B (4).

Based on a partial 16S rRNA sequence, it was determined that strain QW90 was phylogentically related to the *Bacillus* genus. However, based on some phenotypic characteristics it was determined that it was sufficiently different from other known species of *Bacillus*.

The isolated bacteria were pink to red when supplemented with selenite. This was due to the accumulation of elemental selenium and was an indication that selenite was reduced. Roux *et al* also observed such red, round bodies within the cells of *Ralstonia metallidurans* CH34 as elemental selenium (18).

The biological methods and the effects of different environmental parameters were used to demonstrate the removal of sodium selenite by strain QW90. The results obtained showed that under the following conditions a maximum removal of sodium selenite in the supernatant from 800 µg/ml to 0 occurred after 2 days: pH 7.0, temperature 30 °C, and 150 rpm. Generally, there was a good correlation between the optimal pH, temperature, and rpm for the growth and removal of selenite by strain QW90.

Transformation of selenite to elemental selenium could offer an important mechanism for the removal of toxic selenite from polluted environments. Therefore, this strain may be a good candidate for bioremediation of highly polluted effluents from industrial operations. Conventional chemical methods to remove toxic oxyanions are expensive and require more energy or large quantities of chemical reagents, while microbial reduction of these toxic oxyanions is cost effective and supports green technology. In addition, any bacteria capable of reducing selenite could be useful in applied biometallurgy of selenite. Finally, rare and expensive metals can be used extensively for their properties as semiconductors (6).

Conclusion

This study showed that the utilization of enrichment culture technique in comparison to the direct plating on agar leads to better isolation of selenite resistant bacteria. Bacterial strain was resistant to high concentrations of selenite and also, it reduced selenite to red elemental selenium. Therefore, this microorganism could be further used for bioremediation of contaminated sites.

Ethical issues

This study was approved by Islamic Azad University, Qom branch.

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