Chemosphere 156 (2016) 69–75

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Evaluation of chromate reductase activity in the cell-free culture filtrate of *Arthrobacter* sp. SUK 1201 isolated from chromite mine overburden

Satarupa Dey, A.K. Paul*

Microbiology Laboratory, Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata, 700 019, India

HIGHLIGHTS

- First report of extracellular Cr(VI) reductase production by *Arthrobacter* sp.isolated from chromite mine overburden.
- Extracellular chromate reductase production by the isolate is inducible in nature.
- K_m and V_{max} of the extracellular chromate reductase indicated its high affinity to Cr(VI).
- Activation energy (Ea) of the extracellular chromate reductase was found to be 36.209 kJ mol^{-1}.
- Extracellular reductase could be effectively utilized to reduce Cr(VI) in mine effluents.

ARTICLE INFO

Article history:
Received 4 November 2015
Received in revised form
5 April 2016
Accepted 24 April 2016
Available online 10 May 2016

Handling Editor: Martine Leermakers

Keywords:
Arthrobacter
Chromite mine overburden
Cell-free culture filtrate
Extracellular chromate reductase
Chromium bioremediation
Mining waste water

ABSTRACT

Arthrobacter sp. SUK 1201, a chromate resistant and reducing bacterium isolated from chromite mine overburden of Sukinda valley, Odisha, India has been evaluated for its hexavalent chromium [Cr(VI)] reduction potential using cell-free culture filtrate as extracellular chromate reductase enzyme. Production of the enzyme was enhanced in presence of Cr(VI) and its reducing efficiency was increased with increasing concentration of Cr(VI). The Michaelis-Menten constant (K_m) and the maximum specific velocity (V_{max}) of the extracellular Cr(VI) reductase were calculated to be 54.03 μM Cr(VI) and 5.803 U mg^{-1} of protein respectively showing high affinity towards Cr(VI). The reducing activity of the enzyme was maximum at pH 6.5–7.5 and at a temperature of 35 °C and was dependent on NADH. The enzyme was tolerant to different metals such as Mn(II), Mg(II) and Fe(III) and was able to reduce Cr(VI) present in chromite mine seepage. These findings suggest that the extracellular chromate reductase of *Arthrobacter* sp. SUK 1201 has a great promise for use in Cr(VI) detoxification under different environmental conditions, particularly in the mining waste water treatment systems.

1. Introduction

Hexavalent chromium [Cr(VI)] is a strong oxidizer, which exists as hydrochromate ($HCrO^{-4}$), chromate (CrO^{-2}) or dichromate ($Cr_2O_7^{-2}$) and is highly soluble at neutral pH. In nature, Cr(VI) is formed as water soluble anions or neutral species (Kumaresan and Riyazuddin, 1999) and is considered as one of the 17 chemicals posing great threat to human health and environment (Marsh and Mc Inerney, 2001). It is readily taken up by the cell via sulphate pathway because of its structural similarity to SO_4. Toxicity of Cr(VI) is mainly attributed to the process of reduction of Cr(VI) to lower oxidation state, leading to the formation of Cr(V) and reactive oxygen species (ROS) causing ultimately to cellular damage (Cervantes et al., 2001; Ackerley et al., 2004). Such damaging actions may be due to defective replication, transcription, sister chromatid exchange, chromosomal aberrations, cell transformation (Dayan and Paine, 2001) and mutations leading to carcinogenic effect (McLean and Beveridge, 2001).

Microbial reduction of toxic Cr(VI) has been identified as a bioremediation tool not only to detoxify chromium, but also to recover the non toxic Cr(III) by physical means. Chromate reductase, the central enzyme involved in bioreduction of Cr(VI) to Cr(III),

* Corresponding author.
E-mail addresses: dey1919@gmail.com (S. Dey), amalk_paul@yahoo.co.in (A.K. Paul).

http://dx.doi.org/10.1016/j.chemosphere.2016.04.101
0045-6535/

has been described as an intracellular one in several bacteria (Camargo et al., 2004; Pal et al., 2005; Opperman et al., 2008). The extracellular chromate reductases, on the other hand are advantageous to the cells as they protect the cells from toxic Cr(VI), prevent the entry of insoluble Cr(III) into the cells and damages to DNA. However, reports of extracellular Cr(VI) reductase enzymes from chromate reducing bacteria are few (Gnanamani et al., 2010; Rath et al., 2014; Mala et al., 2015).

Extracellular chromate reductases are usually produced during growth of the bacteria in presence (Mala et al., 2015) or in absence of Cr(VI) and are released into the medium where they carry out the Cr(VI) reduction process. The reduced product, Cr(III) being insoluble at neutral pH is usually precipitated in the environment. Priester et al. (2006) have demonstrated that chromate reductase produced in the cytoplasm of *Pseudomonas putida* is released in to the external environment following cell lysis or secretion and reduced Cr(VI) extracellularly resulting precipitation of Cr(III) outside the cell. Similarly, McLean and Beveridge (2001) have reported the extracellular chromate reductase activity in soil pseudomonads. Smith and Gadd (2000) also established the extracellular Cr(VI) reduction in sulphate reducing bacteria and 90% of the reduced Cr was detected in the supernatant. Wang et al. (1991) reported that bacteria with membrane bound reductases can also reduce Cr(VI) extracellularly. More recently, extracellular chromate reductase activities, particularly of *Bacillus* spp. have been demonstrated by several others (Gnanamani et al., 2010; Rath et al., 2014; Mala et al., 2015).

During the course of our survey for bacterial strains capable of tolerating and reducing high concentrations of Cr(VI) from chromite mine overburden, we have isolated a potent chromate reducing strain *Arthrobacter* sp. SUK 1201 (MTCC 8728, Genbank Accession No. JQ312665). The strain was capable of reducing Cr(VI) during growth (Dey and Paul, 2012), by free whole cells (Dey and Paul, 2014b) as well as immobilized whole cells (Dey and Paul, 2014a) leading to formation and extracellular precipitation of Cr(III). Although preliminary work on the optimization of intracellular crude chromate reductase enzyme of SUK 1201 (Dey and Paul, 2013a) has been done, no attempt has so far been made to characterize the chromate reducing ability of the extracellular enzyme produced by this isolate. The present study, therefore, appears to be the first report on the evaluation of Cr(VI) reduction potential of the crude extracellular enzyme obtained from actively growing culture of *Arthrobacter* sp. SUK 1201. The conditions for Cr(VI) reduction by crude extracellular enzyme (the cell-free culture filtrate) have been optimized and the ability of the enzyme to reduce Cr(VI) in mine effluent has also been evaluated.

2. Materials and methods

Arthrobacter sp. SUK 1201, (MTCC Accession No. 8728 and NCBI Genbank Accession No. JQ312665) the chromium resistant and reducing Gram-positive bacterium isolated from chromite mine overburden of Sukinda valley, Odisha, India (Dey and Paul, 2013a) was used throughout this study. The strain was maintained on peptone-yeast extract-glucose (PYEG) agar medium (Wang and Xiao, 1995) supplemented with 2 mM Cr(VI) and the over-night grown cultures on slopes of PYEG were stored at 4 °C for short term preservation.

2.1. Preparation of inoculum

The strain was grown in PYEG medium supplemented with 2 mM Cr(VI) for 24 h at 35 °C under continuous shaking (120 rpm). Cells were harvested by centrifugation at 10,000 rpm for 10 min at 4 °C under aseptic condition, washed 2–3 times with sterile, ice cold 10 mM Tris buffer at pH 7.0 and suspended in the same buffer to use as inoculum.

2.2. Production of extracellular enzyme

For production of extracellular enzyme, mineral salts (MS) medium (Wang and Xiao, 1995) supplemented with 700 μM Cr (VI) was inoculated with freshly prepared cell suspension as described above and the inoculum density was maintained at10^7 cells mL^{-1}. The flasks were incubated at 35 °C in a rotary shaker (120 rpm) and samples were withdrawn at regular intervals. The culture, after 72 h of growth was subjected to centrifugation at 10,000 rpm for 20 min at 4 °C. The cell–free culture filtrate containing 418 μM of residual Cr(VI) was used as the source of crude extracellular chromate reductase.

2.3. Assay of extracellular chromate reductase

Chromate reductase activity of the cell-free culture filtrate was assayed following the procedure as described by Park et al. (2000). The assay mixture (1.0 mL) contained 100 μM Cr(VI) (as K_2CrO_4) in 0.2 M Tris buffer (pH 7.0), 0.1 mM NADH and the reaction was initiated by adding 0.2 mL of cell-free culture filtrate as extracellular chromate reductase. Cr(VI) reduction was measured by estimating the decrease in Cr(VI) in the reaction mixture after 30 min of incubation at 35 °C. The reaction was stopped by addition of 0.5 mL of 20% TCA and the residual Cr(VI) was estimated using standard diphenylcarbazide method (Park et al., 2000). A control set was prepared in similar manner but with heat inactivated culture filtrate. Total chromium was measured using a Varian Atomic Absorption Spectrometer (SpectrAA-20Plus).

One unit (U) of Cr(VI) reductase activity was defined as the amount of enzyme that convert 1.0 μM Cr(VI) per min at 35 °C. Specific activity of chromate reductase was expressed as U mg^{-1} of protein. Protein content of the cell-free culture filtrate was measured following the standard method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

2.4. Characterization of chromate reductase

The constitutive and inducible nature of crude extracellular chromate reductase was determined by growing the organism in absence and in the presence of 700 μM Cr(VI) during growth. In addition, effect of electron donors, pH, temperature, metal ions, inhibitors and kinetics of extracellular chromate reductase activity were determined following the standard procedures.

2.5. Application in mine effluent treatment

Chromite mine effluents have been reported to contain 60 μM of Cr(VI) in addition to number of other soluble metal ions in different concentrations. In order to find suitable application of extracellular chromate reductase, the mine effluent was treated with different concentration of cell-free culture filtrate as the crude extracellular enzyme following the method as described in Section 2.2. The assay mixture consist of mine effluent [60 μM Cr(VI)], 0.1–0.4 mL cell-free culture filtrate as extracellular chromate reductase and 0.1 mM NADH. The residual Cr(VI) in the effluent was determined after 30 and 60 min of incubation.

2.6. Statistical analysis

All experiments were carried out in triplicates and the results were expressed as mean ± S.D.