Environmental Technology & Innovation 6 (2016) 94–104

Contents lists available at ScienceDirect

Environmental Technology & Innovation

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

Mercury resistance and volatilization by *Pseudoxanthomonas* sp. SE1 isolated from soil

Khandaker Rayhan Mahbub *, Kannan Krishnan, Ravi Naidu, Mallavarapu Megharaj

Global Centre for Environmental Remediation, Faculty of Science and Information Technology, The University of Newcastle, Callaghan NSW 2308, Australia
Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC-CARE), Mawson Lakes, Adelaide SA 5095, Australia

HIGHLIGHTS

- *Pseudoxanthomonas* sp. SE1 can resist and volatilize inorganic Hg from solution.
- The isolated bacterial strain harboured mercuric reductase gene and produced mercuric reductase enzyme.
- Demonstration of volatilization of Hg from solution due to bacterial reduction.
- The strain is a potential Hg-remediation bio-agent.

ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form 31 July 2016
Accepted 8 August 2016
Available online 12 August 2016

Keywords:
Mercury
Volatilization
Bioremediation
merA
Pseudoxanthomonas

ABSTRACT

A mercury resistant bacterial strain SE1 isolated from contaminated soil was identified as *Pseudoxanthomonas* based on 16s rRNA sequencing. The Hg resistance was examined in both nutrient-rich media as well as low nutrient media and expressed as EC_{50} and MIC values. Estimated EC_{50} and MIC values in nutrient-rich media and low nutrient media had the following respective recordings — 22.6 mg L^{-1}; 23.1 mg L^{-1} and 1.4 mg L^{-1} and 1.7 mg L^{-1}. The isolate was able to volatilize inorganic mercury demonstrated by a modified photographic film experiment and subsequently revealed its ability to remove mercury from the solution. The ICP-QQQ-MS analysis of SE1 inoculated solution showed almost 60% of 1.5 mg L^{-1} mercury was volatilized in 6 h and almost 40% were accumulated in cell pellets. The mercuric reductase gene *merA* was identified in the genome of isolate SE1 and sequenced. The deduced amino acid sequence of *merA* gene indicated a sequence homology with different organisms from the alpha proteobacteria group and eukaryotic fungi. *merA* encoded enzyme mercuric reductase activity was evident in the crude protein of the isolate. The isolate's ability to resist Hg, it's Hg volatilization potential and the presence of *merA* gene and mercuric reductase enzyme demonstrates the potential application of this strain in mercury bioremediation.

* Correspondence to: Global Centre for Environmental Remediation, ATC Building, Level-1, Faculty of Science and Information Technology, The University of Newcastle, Callaghan, NSW 2308, Australia.

E-mail addresses: KhandakerRayhan.Mahbub@uon.edu.au, krmjissan@gmail.com (K.R. Mahbub), kannan.krishnan@newcastle.edu.au (K. Krishnan), ravi.naidu@crccare.com, ravi.naidu@newcastle.edu.au (R. Naidu), megh.mallavarapu@newcastle.edu.au (M. Megharaj).

http://dx.doi.org/10.1016/j.eti.2016.08.001
2352-1864/

1. Introduction

Because of its persistent nature and accumulation in the food web, mercury (Hg) is considered one of the most toxic heavy metals. The major sources of Hg are natural in character (5000 MG/year), although anthropogenic activities contribute a considerable amount (2320 MG/year) to the global Hg pool (Pirrone et al., 2010). The main sources of mercury pollution are artisanal and small scale gold mining, combustion of coal and fossil fuel, production of non-ferrous metals and disposal of Hg contaminated wastes (Mason et al., 2012). The other significant sources of anthropogenic mercury are chlor-alkali plants, discarded thermometers, batteries and fluorescent lamps, cement production and use of agricultural pesticides and fertilizers containing mercury (Xu et al., 2015). Emissions from all these sources result in elevated levels of mercury in the adjacent soils and waters which are detrimental to the inhabiting micro and macro population. Hg can also travel through the atmosphere and be exposed to an environment which is geographically far from the pollution source (Nelson et al., 2012). Elemental Hg (Hg^0) is considered to be non-toxic or less toxic, whereas inorganic and organic forms of Hg can be toxic to the environment. Hg toxicity leads to a number of clinical conditions in humans and other terrestrial and aquatic organisms (Bernhoft, 2011; Bose-O'Reilly et al., 2010; Chen and Yang, 2012; Houston, 2011).

To eradicate the Hg pollution problem, different clean up technologies have been proposed. Hg cannot be converted into a nontoxic compound; rather the toxic ionic and organic forms can be transformed into less toxic species such as elemental Hg or Hg sulphides which are less poisonous and do not enter the food chain. The traditional physicochemical processes of Hg remediation are as follows: shifting or disposal of Hg contaminated substances to a remote area, solidification and stabilization, amalgamation, soil washing, acid extraction, thermal treatment, vitrification, precipitation, adsorption and membrane filtration (Wang et al., 2012; Wilkerson et al., 2013). These technologies are not environment friendly and are expensive (Wagner-Döbler, 2013). Alternatively, the microbial remediation of Hg may offer a greener and cheaper technology that removes toxic forms of Hg from contaminated point sources.

Hg resistant bacteria have been extensively studied in previous decades. These bacteria have a cytoplasmic enzyme "mercuric reductase" which catalyses the reduction of soluble Hg^{2+} to volatile elemental Hg^0 that subsequently diffuses from the cell (Wagner-Döbler, 2003). Volatilization of Hg from bacterial cells is a well-known resistance mechanism and the genetic determinant — the "*mer*" operon is a typical genetic system (Felske et al., 2003; Nies, 1999; Summers and Lewis, 1973). Instead of being volatilized and released back to atmosphere, the reduced insoluble metallic Hg can be accumulated in a bioreactor (Wagner-Döbler, 2003). It has been reported that adsorption or sequestering of Hg by cell surface components of living microorganisms as well as dead cells occurs (Ahluwalia and Goyal, 2007; François et al., 2012). In certain conditions some resistant bacteria can secrete exo-polymers that adsorb Hg^{2+}. Precipitation of Hg^{2+} as insoluble HgS (cinnabar) is a potential bioremediation technology in aerobic conditions but not in anaerobic conditions, because in anoxic environment the precipitated HgS is taken up by sulphate reducing bacteria and is then methylated (Lefebvre et al., 2007).

Keeping the bioremediation potential in mind, the present study focused on: (i) isolation of a Hg resistant bacterium from Hg contaminated soil and its phylogenetic analysis; (ii) Hg toxicity study on the isolated bacteria in both minimal and nutrient rich media; (iii) the isolate's Hg volatilization capability; (iv) evaluating the isolate's ability to remove Hg; (v) determining mercuric reductase enzyme activity; and (vi) identifying the "*merA*" gene.

2. Materials and methods

2.1. Isolation of mercury resistant bacterium

Isolation of Hg resistant bacterium was done in Hg spiked low nutrient medium which was referred to low phosphate (LP) media. The Hg contaminated soil sample was collected from New South Wales, Australia. The composition of the LP media is as follows (Mahbub et al., 2016; Rathnayake et al., 2013): 0.195% 2-(N-morpholino) ethanesulfonic acid; 0.001% Na_2HPO_4; 0.005% NH_4Cl; 0.002% KCl; 0.024% $MgSO_4 \cdot 7H_2O$; 0.014% $CaSO_4$; 0.0004% $FeSO_4 \cdot 7H_2O$; 0.1% SL7 trace solution (0.1% HCl, 35 mg L^{-1} $ZnCl_2$, 50 mg L^{-1} $MnCl_2 \cdot H_2O$, 30 mg L^{-1} H_3BO_3, 200 mg $CoCl_2 \cdot 2H_2O$, 20 mg L^{-1} $CuCl_2 \cdot 2H_2O$, 20 mg L^{-1} $NiCl_2 \cdot 6H_2O$, 40 mg L^{-1} $NaMoO \cdot 2H_2O$) and 0.2% glucose. The media pH was adjusted to 6.4 and was sterilized by autoclaving. After autoclaving 10 ml of aliquots was supplemented with filter sterilized Hg (supplied as $Hg(NO_3)_2$) to a final concentration of 3 mg L^{-1}. This Hg supplemented media was then inoculated with 1 g contaminated soil and incubated in the dark for 5 days at 25 °C on a rotary shaker (120 rpm). After 5 d of incubation 1 ml aliquot was transferred to a fresh media of the same composition and incubated for 3 days at 25 °C. Then a single colony was isolated on a 3 mg L^{-1} Hg supplemented LP-agar plate, preserved in 15% glycerol broth at −80 °C.

2.2. Sequencing of 16s rRNA gene

Identification of the isolated Hg resistant bacterial strain was carried out using PCR amplification of 16s rRNA gene and then sequencing the amplified product. Bacterial DNA was extracted using a genomic DNA prep kit (ISOLATE II genomic DNA kit, Bioline). A 50 μl reaction mixture was prepared containing: 10 ng DNA template, 10 μM universal primer mix (E8F: 5-AGAGTTTGATCCTGGCTCAG-3 and 1541R: 5-AAGGAGGTGATCCANCCRCA-3; PCR product size 1500 bp) and 25 μl Mango mix (Mango Mix™, Bioline) composed of PCR buffer, $MgCl_2$, dNTPs and Taq polymerase. DNA template control, forward