# Characterization of Arsenic Oxidation and Chromium Reduction Potential of *Micrococcus* Sp. with Plant Growth Promoting Traits

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**Abstract—***Biological nitrogen fixation (BNF) is very susceptible to environmental fluctuations. It is still arguable how BNF is regulated under stress conditions especially arsenic (As) and chromium (Cr) contaminated agricultural fields. The capability to colonize the rhizosphere of plants in metal contaminated soils is highly important for symbiotic and free-living plant growth-promoting microorganisms used as inoculants, since they can contribute to enhance metal tolerance, speciation of metal and limit metal uptake by plants. The aim of this study was to find bacteria which have PGPR activity and soil detoxification attributes as well. We isolated a bacterium which had nitrogen fixing ability, produced indole acetic acid utilizing tryptophan as a substrate. It could resist 8.4 mM arsenite and >650 mM arsenate and >2 mM Cr with oxidation of arsenite. It was reported that isolates those were more resistant to Cr were not necessarily reduce more Cr, but our selected strain reduced ~8 mg Cr(VI) $g^{-1}$ dry cell biomass. Molecular character-ization followed by phylogenetic analysis revealed that the isolate is Micrococcus sp. The behaviour of this isolate under As and Cr stress indicated that it might be able to colonize efficiently in As and/or Cr contaminated soils, promote growth of different plant species and detoxify contaminated environments.*

**Keywords***: Rhizosphere, PGPR, Chromium reduction, Arsenic oxidation, Bioremediation.*

## 1. INTRODUCTION

Contamination of arsenic and chromium in agricultural land is of serious concern as they enter into food chain through plant uptake and reduce soil fertility [1]. The mobility, toxicity, and environmental fate of arsenic and chromium in soil are largely dependent on their redox speciation [2, 3]. It is evident that microorganisms in the soil take part in As and Cr speciation. As(III) and Cr(VI) are far more mobile, soluble and hence toxic than As(V) and Cr(III). Nitrogen input through BNF is the major rout of soil nitrogen. Toxicity due to As and Cr contamination might be the cause of fluctuations of nitrogen fixing microorganisms and thereby nitrogen input in soil. Regulation of BNF under stress condi- tions especially arsenic (As) and chromium (Cr) contaminated agricultural fields is still debatable [4]. Hence the study of colonization of rhizosperic soil by symbiotic and free-living microorganisms in these metal contaminated soils is highly important since they can contribute in plant growth, enhance metal tolerance, control speciation of metal and limit metal uptake by plants [5]. The aim of this study was to find bacteria which have PGPR activity and soil detoxification attributes as well.

## 2. MATERIALS AND METHODS

### 2.1 Microorganism and culture condition

A bacterium isolated from tannery waste water contaminated soil was taken for this study. The isolate was routinely maintained in mineral salt medium (MSM) with the following composition (g $L^{-1}$), NaCl-4.68; $NH_4Cl$-1.07; KCl-1.49; $Na_2SO_4$-0.43; $MgCl_2$, $6H_2O$-0.2; $CaCl_2$, $2H_2O$-0.03) supplemented with glycerol phosphate (2 mM), dextrose (0.05%), yeast extracts (0.1%) at 30°C.

### 2.2 Chromium (VI) reduction assay

Chromium reduction ability of the isolate was studied with spectrophotometric technique using standard S-diphenylcarbazide (DPC) [6]. Isolate was grown in presence of 2 ppm Cr(VI) in MSM medium. The culture soup was used in reduction assay. Chromium reduction assay was carried out by adding 125 µl of DPC reagent (100 ml DPC reagent was prepared by mixing two solutions, solution 1 (24 ml of 85% $H_3PO_4$ in 56 ml distilled water) and solution 2 (76 mg DPC in 20 ml 95% ethanol)) to 1 ml of test samples, The absorbance of the treatment was measured by spectrophotometer at 540 nm wavelength after 20 minute incubation.

### 2.3 As(III) oxidation assay

The isolated strain was tested for its As(III) oxidation ability by using silver nitrate ($AgNO_3$) screening method. The strain was grown on Luria Bertani broth with or witout 1 mM As(III) for 4 days at 30°C. The culture supernatant was collected by centrifugation and assayed with $AgNO_3$ solution (0.1 M). Formation of brown precipitate in supernatant indicated positive As(III) oxidation reaction [7].

### 2.4 Indole acetic acid (IAA) production by the isolate

The Cr resistant isolate was grown in MSM in presence of 0.5 mg $mL^{-1}$ L-tryptophan. After 5 days incubation at 30°C, 0.5 mL of cell supernatent was assayed after centrifugation with the addition of 25 µL of 10 mM ortho-phosphoric acid and 1 mL of Salkowski's reagent (2% 0.5 M $FeCl_3$ in 35% perchloric acid) and incubation for 45 minutes for the pink colour to develop [8].

### 2.5 Nitrogen fixation ability

The isolates was inoculated into Yeast Extract Manitol Agar (YEMA) [$K_2HPO_4$ 0.5 g, $K_2SO_2,7H_2O$ 0.2g, NaCl 0.1 g, manitol 10 g, yeast extract 1 g, agar 20 g, water 1000 ml] medium containing bromothymal blue (1% dissolved in ethanol). After 48 h of incubation the plates are observed.

## 3. RESULTS AND DISCUSSION

### 3.1 Chromium reduction by the isolate

The selected bacteria showed high chromium reduction potential while growing in 2 mM Cr(VI). During growth the bacteria reduces more than 8210 µg Cr(VI) in 48 h while the reduction increase more than 8218 µg in 60 h (Fig. 1).

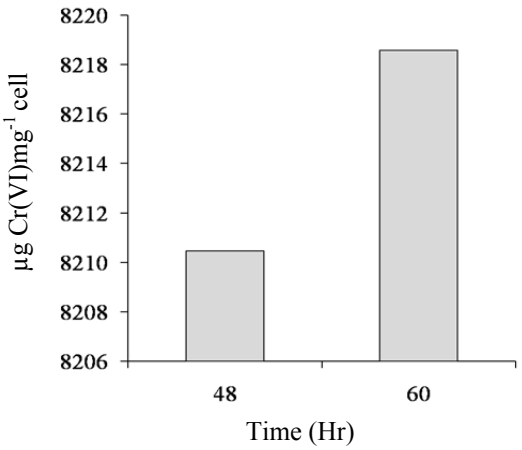


**Fig. 1: Reduction of Cr(VI) in 48 h and 60 h. The result is expressed in µg Cr(VI) $mg^{-1}$ cell dry cell.**

### 3.2 As(III) oxidation by the isolate

The result of silver nitrate assay is presented in Fig.2. Both As free culture supernatant (A) and medium incubated with arsenite (B) did not showed brown precipitation. But, the same concentration of arsenite, when incubated with cell, it forms brown precipitation (D). To determine how much arsenite converted to arsenate, graded concentration of arsenate was incubated and assayed (C). This assay clearly indicated that the selected bacteria could significantly oxidize As(III).

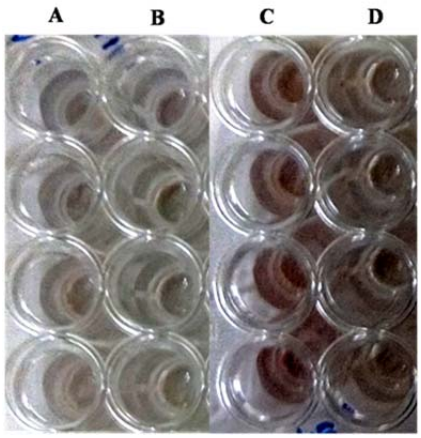


**Fig. 2: Silver nitrate test, (A) Culture supernatant, (B) Medium with As(III), (C) Medium with As(V) and (D) Culture supernatant with As(III).**

### 3.3 IAA production by the isolate

This assay was performed in presence or absence of As(III). Appearance of pink colour indicated presence of IAA (Fig. 3). No pink colour was observed in medium (A) or medium amended with As(III) (B). While dense pink colour formed with culture supernatant in presence or absence of As indicated bacteria efficiently produce IAA like molecule and As did not interfere on its production.

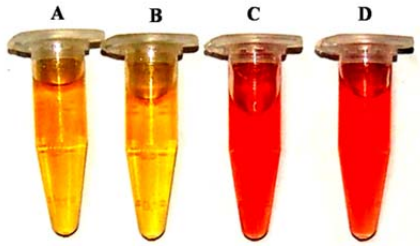


**Fig. 3: IAA assay, (A) Medium, (B) Medium+As(III), (C) Cell supernatant and (D) Cell supernatant with As(III).**

### 3.4 Nitrogen fixation by the bacteria

The isolated bacteria on bromothymol blue containing medium produce yellow colony indicated it produces acid that lowered the pH (Fig. 4). It might be due to production of ammonium ion which is produced during nitrogen fixation. We also observed that this isolate could grow in nitrogen free medium.

**Fig. 4: Growth of *Micrococcus* sp. on YEM agar medium containing bromothymol blue.**

## 4. CONCLUSIONS

The selected tannery isolates showed Cr(VI) resistant and arsenic oxidation ability. Isolate is identified as *Micrococcus* sp. Apart from Cr(VI) reduction and As oxidation it could produce IAA and might be able to nitrogen fixation. The behaviour of this isolate under As and Cr stress indicated that it might be able to colonize efficiently in As and/or Cr contaminated soils, promote growth of different plant species and detoxify contaminated environments.

## 5. ACKNOWLEDGEMENTS

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