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### EVALUATION OF IAA AND PHAs PRODUCTION BY CHROMIUM RESISTANT BACTERIAL ISOLATES

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#### KEYWORDS

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#### ABSTRACT

Extensive chromite mining activities have generated huge amount of toxic hexavalent chromium Cr (VI), which persists in the soil for many years. Long term accumulation of Cr(VI) in the soil decreases crop productivity in adjoining farming land. On account of that, 14 chromium resistant bacteria (CRB) were subjected to Indole Acetic Acid (IAA) and Polyhydroxyalkanoates (PHAs) Production. Bacterial strains such as *Bacillus* sp. CTSI-07, *Enterobacter* sp. CTWI-06 and *Acinetobacter* sp. CTWI-07 were producing 24, 114 and 106 µg/ml of IAA respectively. In addition, these bacterial isolates produced 0.75, 0.30 and 0.42 g/l of PHAs under submerged fermentation process. Moreover, higher amount of PHAs production (21.42%) was exhibited by *Bacillus* sp. CTSI-07. The extracted biopolymer is polyhydroxybutyrate (PHB) (most common homopolymer of PHAs) as revealed from structural characterization. As these bacterial strains have the capability to produce IAA and PHAs, which may be utilized for long term bioremediation of Cr(VI) in chromium contaminated soil as well as to maintain soil fertility.

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## 1 Introduction

Extensive chromite mining activities have generated huge amount of toxic waste that causes environmental pollution and public health hazard. These waste material contains a wide array of toxic metal predominantly hexavalent chromium Cr(VI), which persists in the soil for many years (Samantaray & Mishra, 2012). Long term accumulation of Cr(VI) in the soil decreases crop productivity in adjoining farming land and can effectively impact on the microbial diversity (Yu et al., 2014). As the conventional methods for treatment of Cr(VI) are not environmental friendly, thus it is imperative to look into economic and ecofriendly alternatives. In this context, the prime concern is to reclaim and restore soil properties through the process of microbial bioremediation (Upadhyay et al., 2016). Microbes like bacteria are playing a vital role in maintaining physical and chemical structure of soil as well as its fertility (Ahemad, 2015). Bacteria respond quickly and are sensitive to subtle environmental changes; as a matter of fact, they have been considered as bio-indicators of soil texture (Valverde et al., 2011).

Bacterial strains showing higher tolerance or resistance to Cr(VI) as well as nitrogen fixation, phosphate and potassium solubilization (NPK) capability are the potential candidate for bioremediation of Cr(VI) contaminated soil (Ahemad, 2015). In addition to Cr(VI) detoxification, chromium resistant bacterial strains are also increasing nutrient availability of chromium contaminated soils (Pattnaik et al., 2017). In this regard, several studies have reported the utilization of plant growth-promoting bacteria (PGPB) that solubilize phosphate and synthesize growth-promoting substances such as indoleacetic acid (IAA) can be applied for bioremediation of metal-contaminated soil (Yu et al., 2014; Pattnaik et al., 2017; Patel et al., 2017). Application of PGPB for Cr(VI) bioremediation not only increase NPK and IAA availability in chromium contaminated soil but also stimulate seed germination (Yu et al., 2014), seedling roots (Patten & Glick, 2002) and inhibition of phytopathogens (Haas & D efago, 2005). Additionally, utilization of PGPB strains with PHAs synthesizing ability for reclamation of chromium contaminated soil may be an added advantage. PHAs are the carbon and energy storage granule present in the cytosol of the bacteria (Mohapatra et al., 2014) and enhances survivability of cells during unfavorable environmental conditions such as carbon starvation, exposure to radiation, desiccation, toxic metals & oxidants (Mohapatra et al., 2016; Koller, 2017). This may increase the shelf life period of bacterial bioremediation of Cr(VI) in chromium contaminated soil. In light of above, the present research is aimed to study the IAA and PHAs production by Cr(VI) resistant or tolerant bacteria isolated from Sukinda mining area.

## 2 Materials and Methods

### 2.1 Selection of source organism

In preceding work 14 chromium resistant (up to 3500ppm) bacteria (CRB) were isolated from the soil, sediment & water samples of Sukinda mining area and preserved in glycerol stock at -80°C. In the present study, these bacterial isolates were revived in the Luria Bertani (LB) agar medium and subjected to Indole Acetic Acid (IAA) and Polyhydroxyalkanoates (PHAs) production. All the culture media and chemicals used in the research work were procured from Hi-Media Laboratories Pvt. Ltd. Moreover, the standard and organic solvents used in this research work were procured from Merck bioscience and Sigma-Aldrich chemicals respectively.

### 2.2 Estimation of IAA production by CRB

IAA production by the Cr(VI) resistant bacterial strains was carried out using the Salkowski's method (Mohite, 2013). Screened bacterial isolates were grown in peptone water (Himedia, India) and incubated at 30°C for 4 days. After incubation, the cultivated medium was centrifuged at 10000rpm for 12min. One ml of supernatant was collected and mixed with Salkowski reagent (2% 0.5M FeCl<sub>3</sub> in 35% HClO<sub>4</sub>), kept at room temperature. Then, the quantitative estimation of IAA was conducted by measuring optical density at 540nm after 30 min and 120 min intervals.

### 2.3 Screening of CRB for PHAs production

Sudan black B staining was conducted for detection of presence of the PHAs granule in cytoplasm of the bacterial cells (Mohapatra et al., 2015). Before screening, the isolates were induced to accumulate PHAs by growing in the growth medium (GM) that contained L-glutamic acid (3.8 g/L<sup>-1</sup>), malic acid (2.7 g/L<sup>-1</sup>), yeast extract (2.0 g/L<sup>-1</sup>), KH<sub>2</sub>PO<sub>4</sub> (0.5 g/L<sup>-1</sup>), K<sub>2</sub>HPO<sub>4</sub> (0.5 g/L<sup>-1</sup>), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.8 g/L<sup>-1</sup>), Mg SO<sub>4</sub>·7H<sub>2</sub>O (0.2 g/L<sup>-1</sup>), CaCl<sub>2</sub>·2H<sub>2</sub>O (0.053 g/L<sup>-1</sup>), MnSO<sub>4</sub>·5H<sub>2</sub>O (0.001 g/L<sup>-1</sup>), NaCl (5.0 g/L<sup>-1</sup>), nicotinic acid (1.0 mg/L<sup>-1</sup>), thiamine (1.0 mg/L<sup>-1</sup>), biotine (0.01 mg/L<sup>-1</sup>), glucose (10.0 g/L<sup>-1</sup>) medium for 24 h. The bacterial cells were stained with 3% Sudan black B solution for 10 min and decolourized by xylene. Then, the bacteria cells were stained with counter stain safranin for 10 seconds and observed under oil immersion microscope (Leica DM5000B). Further, the bacterial cells were again subjected to Nile red staining (Maity et al., 2017) for confirmation of PHAs granule in the cytoplasm.

### 2.4 PHAs production by CRB

The selected bacterial strains were subjected for PHAs production by submerged fermentation process. One-stage batch cultivation in a shake flask method was carried out for PHAs production (Mohapatra et al., 2017a). Bacterial isolates were grown in the GM at 37°C with 120 rpm for 96 hours. Cell biomass (DCW) was then harvested by centrifugation at 6500g, 10 min and dried at 50°C. Dried cell biomass was treated with sodium hypochlorite to digest the non-PHAs materials. The mixture was then centrifuged and subsequently washed twice with acetone, methanol

and diethyl ether (1:1:1) to remove sodium hypochlorite and debris. The partially purified PHAs was dissolved in boiling chloroform to remove the remaining organic solvents and the amount of PHAs was quantified using the formula: % of PHAs production = (Weight of PHAs/ Weight of dry cell biomass) × 100.

## 2.5 Characterization of PHAs by FTIR Analysis

The functional group of extracted PHAs was identified by Fourier transform infrared (FTIR) spectroscopic analysis. Briefly, 2 mg of extracted PHAs film was placed on Attenuated Total Reflectance (ATR) diamond based plate and the IR-spectrum was recorded using a single beam spectrometer (Perkin-Elmer RX I) between wave numbers of 4000 and 400  $\text{cm}^{-1}$  (Dash et al., 2013).

## 3 Results and Discussion

### 3.1 IAA production by Cr(VI) resistant bacteria

Contamination of soil with toxic metal chromium can transform the soil micro-flora and their metabolic activity. These contaminated soils are deficient in several growth promoting nutrients and causes stress

for plants (Upadhyay et al., 2017). Thus, it is the need of the hour to restore soil properties as well as plant growth using microbes. As toxic metal tolerance is one of the vital factors for utilization of native micro-flora in bioremediation. Thus, estimation of Cr(VI) tolerance of 14 bacterial isolates has been carried out in previous study of Pattnaik et al., (2017). In present study, these bacterial isolates were subjected to IAA production using both qualitative and semi-qualitative methods. Among all, bacterial isolates such as *Bacillus* sp. CTSI-07, *Enterobacter* sp. CTWI-06 and *Acinetobacter* sp. CTWI-07 produced approximately 24  $\mu\text{g/ml}$ , 114  $\mu\text{g/ml}$  and 106  $\mu\text{g/ml}$  of IAA respectively (Figure 1; Table 1). In contrast to findings of present study, Upadhaya et al., (2017) reported production of IAA (56.95  $\mu\text{g/ml}$ ) by the chromium resistant bacteria *Bacillus* sp. MNU16 isolated from contaminated coal mining soil. Similar IAA producing metal tolerant bacteria such as *Pantoea stewartii* strain ASI11, *Microbacterium arborescens* strain HU33 and *Enterobacter* sp. strain HU38 were also isolated from rhizospheric soil of *P. juliflora* (Khan et al., 2015). Thus, utilization of these metal resistant bacteria for bioremediation can overcome toxic effect of Cr(VI) as well as increase plant growth (Hansda et al., 2014; Sobariu et al.,

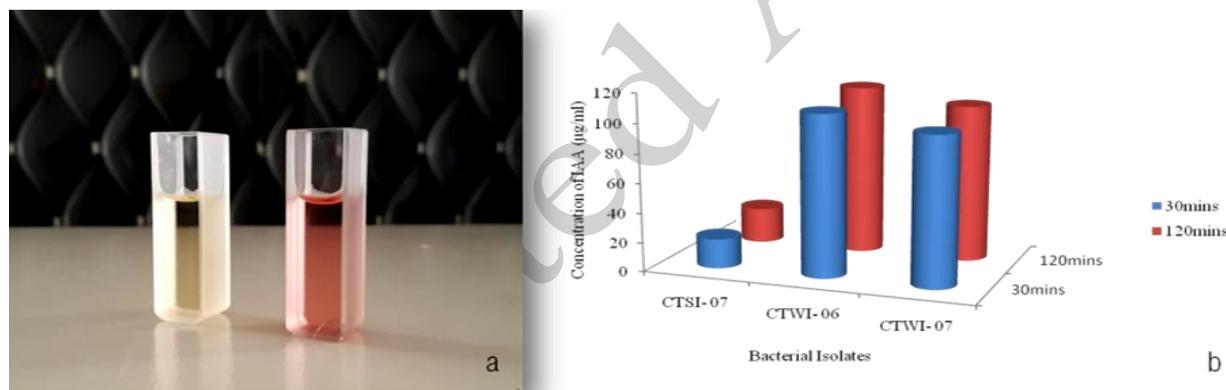


Figure 1 (a) IAA (b) production by Cr(VI) resistant bacterial isolates.

Table 1 IAA and PHAs production by CRB

Isolates Code	IAA Production ( $\mu\text{g/ml}$ )	PHAs Production (g/l)	Isolates Code	IAA Production ( $\mu\text{g/ml}$ )	PHAs Production (g/l)
CTSI-01	-	-	CTWI-01	-	-
CTSI-02	-	-	CTWI-02	-	-
CTSI-03	-	-	CTWI-03	-	-
CTSI-04	-	-	CTWI-04	-	-
CTSI-05	-	-	CTWI-05	-	-
CTSI-06	-	-	CTWI-06	+ / 114 $\mu\text{g/ml}$	+ / 0.30 g/l
CTSI-07	+ / 24 $\mu\text{g/ml}$	+ / 0.75 g/l	CTWI-07	+ / 106 $\mu\text{g/ml}$	+ / 0.42 g/l



1711cm<sup>-1</sup> depicting the (C=O stretch) ester carbonyl group of PHB were also reported by previous workers (Mohapatra et al., 2015; Mohapatra et al., 2017a). Thus, the biopolymer produced by the metal resistant bacteria is PHB as revealed from structural characterization.

### Conclusion

In conclusion, the chromium resistant bacterial isolates such as *Bacillus* sp. CTSI-07, *Enterobacter* sp. CTWI-06 and *Acinetobacter* sp. CTWI-07 have the capability to produce IAA and PHB. These metal resistant bacteria can easily overcome toxic effect of Cr(VI) during bioremediation as well as increase plant growth in the chromium contaminated soil. Moreover, due to their PHAs synthesis ability, they can survive for longer period in unfavorable environmental conditions, which may increase the shelf life period of bioremediation.

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### Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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