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Exploration of NPK Activity Showing Chromium Resistant Bacteria from Sukinda Mining Area

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A B S T R A C T

The goal of the study is to explore potential chromium resistant and nitrogen fixing, phosphate & potassium solubilizing bacteria from Sukinda mining area for bioremediation of Cr (VI) contaminated soil. In toto 25 bacteria were isolated, among them 14 isolates showed resistance to hexavalent chromium. Moreover, bacterial isolates such as *Paenibacillus* sp. CTSI-01, *Micrococcus* sp. CTWI-03 and *Enterobacter* sp. CTWI-06 were able to tolerate 3500ppm of Cr (VI) concentration. The bacterial isolates were affiliated to the genus such as *Bacillus, Paenibacillus, Brevibacillus, Acinetobacter, Enterobacter, Micrococcus and Staphylococcus* by morpho-physiological characterizations. Interestingly, amongst 14 chromium resistant bacteria, three isolates such as *Micrococcus* sp. CTSI-06, *Enterobacter* sp. CTWI-06 and *Acinetobacter* sp. CTWI-07 depicted nitrogen fixation, phosphate and potassium solubilization capability as revealed from solubility index. Thus, these potential chromium resistant and NPK activity showing locally isolated bacterial strains may be used for detoxification of Cr (VI) as well as to increase nutrient availability of chromium contaminated soils.

Introduction

Rapid industrialization coupled with exponential increase in use of toxic chemicals and mining activities has not only resulted in global environmental deterioration, but also has drawn attention of scientists for an effective measure to control pollution. The Sukinda mining zone, Odisha is one of the chromium contaminated area due to chromite mining since more than four decades. As a matter of fact, the flora, fauna along with human population of the mining area are adversely affected due to Cr (VI) pollution (Mishra *et al.*, 2010). Moreover, mining activities are major causes of soil texture transformation and with its biomagnification decreases crop productivity in adjoining farming land (Tripathi *et al.*, 2012, Upadhyaa *et al.*, 2017).

Chromium is a priority pollutant (Mishra *et al.*, 2010) and generally stable in the form of Cr(VI) and Cr(III) in nature. Cr(VI) compounds are highly toxic due to their solubility in water, permeability through cell membranes and subsequently affect protein and nucleic acids of the biological systems as compared to Cr(III) (Wuana and Okieimen, 2011; Sultan and Hasnain, 2005). In addition, Cr(VI) is a well-known mutagenic (Gili *et al.*, 2002) and carcinogenic (Codd *et al.*, 2003)

agent that causes DNA damage (Chen and Hao, 1998) and reduces soil fertility and plant growth (Ahemad, 2015). The conventional methods for treatment of Cr(VI) pollution are not environmental friendly. Thus, it is imperative to look into ecofriendly and economic alternatives where, microbial detoxification seems to be a most plausible approach. Microbes with higher resistance or tolerance to Cr(VI) are the potential candidate for detoxification of Cr(VI). A wide array of bacteria belonging to several genera *Aceinetobacter*, *Serratia*, *Bacillus*, *Pantoea*, *Pseudomonas*, *Staphylococcus*, *Cellulomonas*, *Achromobacter*, *Micrococcus*, *Escherichia*, *Ochrobactrum*, isolated from chromium contaminated sites, have shown biotransformation especially reduction of Cr(VI) to relatively nontoxic Cr(III) (Mishra *et al.,* 2010; Kathiravan *et al.,* 2010).

In this context, the prime concern is to reclaim and restore soil properties through the process of microbial bioremediation (Hansda *et al.,* 2014). Soil is the source of nutrients to the plant and harbors microbes performing various activities essential for maintenance of soil fertility. Such activities of soil microbes are largely affected by pollutants released to the soil, as plant growth is regulated by soil fertility (Hansda *et al.,* 2014). Several studies have reported the utilization of plant growth promoting rhizobacteria (PGPR) for the bioremediation of toxic metals (Samuel *et al.,* 2013; Sobariu *et al.,* 2016; Ndeddy and Babalola, 2016).

However, exploration of efficient Cr(VI) reducing bacteria having phosphate solubilization, nitrogen fixation and potash solubilization activity is a crucial need for the bioremediation of Cr(VI) as well as increasing nutrient availability of contaminated soils. With this background, the present study is envisaged to explore potential chromium resistant or reducing and NPK activity showing bacteria from Sukinda mining area for bioremediation of Cr(VI) contaminated soil.

Materials and Methods

Sampling and physico-chemical parameter analysis

For this study, five different sites, IMFA, Jindal mines, Kamardha mines, Dumsala canal and OMC Ltd. were selected in the Sukinda mining area of district Jajpur, Odisha, located between latitudes 21° 00’ 07” to 21° 02’ 46” N and longitudes 85° 44’ 12” to 85° 47’ 22” E. The water, sediment, sludge and overburden soil samples were collected using sterile containers and processed in the laboratory for physico-chemical parameter and bacteriological analysis. Different physio-chemical parameters such as pH, temperature, moisture, electrical conductivity (EC), total dissolve solid (TDS) were analyzed by standard method. Moreover, total and Cr(VI) content of the samples were analyzed by APHA method using AAS (Das *et al.,* 2013). The chemicals and reagents used in the research work were procured from Hi-Media Laboratories Pvt. Ltd.

Isolation and screening of Cr(VI) resistant bacteria

The aerobic, heterotrophic bacteria were isolated using Lauria Bertani (LB) agar medium supplemented with 100 mg/l of filter (0.22µm) sterilized K_2CrO_4 solution employing serial dilution followed by spread plate technique. The colonies of distinct morphological characters were individually picked up, sub-cultured and preserved in glycerol stock at -80°C for further use. Then, the extent of Cr(VI) tolerance or resistance of bacterial isolates were conducted with increasing (100–3500 mg/l) concentrations of Cr(VI) on LB agar plates and the chromium

tolerance or resistance pattern of bacterial strains were noted down (Mishra *et al.*, 2010).

Morpho-physiological characterization of Cr(VI) resistant bacteria

The morpho-physiological characteristics of Cr(VI) resistance bacterial strains were determined by their colony morphology on LB agar and Gram's reactions with light microscopic imaging. Bacterial isolates were then processed for generic level identification by the standard methods of biochemical, enzymatic, sugar utilization and antibiotic sensitivity tests as prescribed by Bergy's Manual of Determinative Bacteriology (Holt *et al.*, 1994). Then, the obtained results were interpreted with the ABIS online software and the bacterial strains were provisionally identified up to genus level.

Evaluation of N-fixation, phosphate-potassium solubilization of Cr(VI) resistant bacteria

The Cr(VI) resistant bacterial strains were then subjected to nitrogen fixation, phosphate and potassium solubilization. Briefly these bacterial strains were inoculated to nitrogen free medium like Jansen agar medium and incubated at 30±1^{o}C for 72 hours (Pahari and Mishra, 2017). The nitrogen fixing ability of bacterial strains was observed from growth of the bacteria on the medium. Following nitrogen fixation, these bacterial strains were also inoculated to Pikovskaya agar and Aleksandrov agar medium and incubated at 27^{o}C for 7 days.

Then, the halozone formed around colonies were measured and the solubility index (SI) for phosphate (P) and potassium (K) was calculated by the following formulae (Mursyida *et al.*, 2015); Solubility index = [halozone diameter (mm) – colony diameter (mm)] / colony diameter (mm).

Results and Discussion

Physico-chemical parameter analysis

The microbial diversity of chromite mines or contaminated sites depends upon several factors such as pH, temperature, trivalent or hexavalent chromium concentration and rainfall. These microbes have developed the ability to tolerate and reduce chromium and can be used for detoxification of hexavalent chromium contaminated sites. The physico-chemical parameters analysis of the mines sample (Table 1) revels, the pH of water, sediment, sludge and overburden samples were 7.8, 7.32, 7.31, and 7.69 respectively. The pH of water, sediment, sludge and overburden soil samples of Sukinda mining area is almost alkaline in nature. The alkaline pH of chromium contaminated sites due to presence of high amount of chromium, which is generally stable in alkaline pH and hexavalent chromium is dominant in such aqueous environment at pH 6.5 to 9 (Mishra *et al.*, 2010). The hexavalent chromium content in water, sediment, sludge and overburden soil samples were 1.230 mg/L, 713 mg/kg, 350 mg/kg, and 938 mg/kg respectively.

The average hexavalent chromium content of the water samples of the mining area was 1.230 mg/L which far exceeds the prescribed EPA standards of 0.05mg/l (APHA, 2005). The total chromium content of water, sediment, sludge and overburden soil samples were 2.54 mg/L, 6900 mg/kg, 3400 mg/kg, 7300 mg/kg respectively. Moreover, presence of such high levels of hexavalent chromium in the water, sediment, sludge and overburden soil can be attributed to high mining activity vis-a-vis release of untreated waste water from mines, rainwater running off the overburden and dumps collapsing and mixing with water in the river (Das *et al.*, 2013; Mishra *et al.*, 2010).

Isolation and screening of Cr(VI) resistant bacteria

The total aerobic, heterotrophic bacteria in the water and sediment, sludge and overburden soil samples were 8.9 x 10^4 CFU/ml, 8.4 x 10^4 CFU/gm, 2.16 x 10^4 CFU/gm and 1.26 x 10^4 CFU/gm respectively. In to 25 bacteria were isolated, among them 14 isolates showed resistance (Table 2) towards higher concentration of hexavalent chromium. Ten bacterial isolates showed resistance to 1500ppm of hexavalent chromium concentration. Moreover, bacterial isolates such as CTSI-01, CTWI-03 and CTWI-06 were able to tolerate 3500ppm of hexavalent chromium concentration. Such higher level of hexavalent chromium resistance might be due to molecular adaptation of bacterial isolates in the chromium contaminated sites and the difference in resistance pattern indicating to variation of Cr(VI) concentration in the particular area (Mishra *et al.,* 2010; Ilias *et al.,* 2011).

Morpho-physiological characterization of Cr(VI) resistant bacteria

The Gram variability reactions revealed that amongst 14 chromium resistant bacteria, 08 were Gram positive rods and 06 were Gram positive cocci (Table 3). The Cr(VI) resistant bacteria showed urease, lipase and catalase enzymatic activity, however a negative trend in gelatinase and cellulase enzyme activity was observed by these bacterial isolates. Nevertheless, few bacterial isolates showed positive to caseinase, amylase and DNAase enzyme.

Moreover, sugar utilization results of Cr(VI) resistant bacterial isolates concluded that, most of the bacteria were able to assimilate maltose, fructose, dextrose, L-arabinose, mannose, cellobiose and very few bacterial isolates were able to utilized other sugars used in the study.

The bacterial isolates were affiliated to the genus such as *Bacillus, Paenibacillus, Brevibacillus, Acinetobacter, Enterobacter, Micrococcus and Staphylococcus* by morpho-physiological characterizations.

In contrast to our observation, chromium resistant bacteria belonging to the same genera were also reported by most of the researcher (Ankita and Saharan, 2017; Das *et al.,* 2013) while working on Cr (VI) chromium reduction.

Table.1 Physico-chemical parameter analysis of mines sample

Samples	pH	Temp. (^{o}c)	Moisture (%)	EC (µS/cm)	TDS (ppm)	Total Cr (mg/L)	Cr(VI) (mg/L)
Water	7.8	35	--	260	0.10	2.54	1.230
Sediment	7.32	38	12.5	58	0.00	6900	713
Sludge	7.31	35	35	387	0.10	3400	350
OB Soil	7.69	37	25	61	0.00	7300	938

Table.2 Hexavalent chromium tolerance by bacterial isolates

Isolates code	500 ppm	1000 ppm	1500 ppm	2000 ppm	2500 ppm	3000 ppm	3500 ppm
CTSI-01	+++	+++	++	++	++	+	+
CTSI-02	+++	++	+	--	--	--	--
CTSI-03	+	--	--	--	--	--	--
CTSI-04	++	--	--	--	--	--	--
CTSI-05	++	+	--	--	--	--	-
CTSI-06	++	+	--	--	--	--	--
CTSI-07	+++	++	+	--	--	--	--
CTWI-01	+++	++	+	--	--	--	--
CTWI-02	+++	++	+	--	--	--	--
CTWI-03	+++	+++	++	++	++	+	+
CTWI-04	+++	+++	++	++	+	--	--
CTWI-05	+++	+++	++	++	+	--	--
CTWI-06	+++	+++	+++	++	++	+	+
CTWI-07	+++	++	+	+	--	--	--

Table.3 Biochemical characterization of Cr(VI) resistant bacterial isolates

Isolates code	1	2	3	4	5	6	7	8	9	10	11	12	13
CTSI-01	+ve rods	-	+	-	-	+	-	-	+	-	+	+	+
CTSI-02	+ve cocci	-	-	-	-	-	-	-	-	-	-	-	+
CTSI-03	+ve rods	-	-	-	+	-	-	-	-	-	-	+	+
CTSI-04	+ve rods	-	-	-	-	+	-	-	+	+	+	-	+
CTSI-05	+ve rods	-	-	+	-	+	-	-	+	+	+	-	+
CTSI-06	+ve cocci	-	+	-	-	-	-	-	-	-	-	-	+
CTSI-07	+ve rods	-	-	-	+	+	+	-	-	-	-	-	+
CTWI-01	+ve cocci	-	-	-	-	-	-	-	-	+	-	-	+
CTWI-02	+ve rods	-	-	-	-	+	+	-	+	+	+	+	+
CTWI-03	+ve cocci	-	+	+	-	+	+	+	+	-	+	-	+
CTWI-04	+ve rods	-	+	-	-	+	-	-	+	-	-	-	+
CTWI-05	+ve rods	-	+	-	-	+	-	-	-	-	-	+	+
CTWI-06	-ve rods	-	-	+	+	+	+	-	+	-	+	-	+
CTWI-07	+ve cocci	-	+	-	+	-	-	-	-	-	-	-	+

NB: 1. Gram Staining 2. Indole, 3. MR, 4. VP, 5. Citrate, 6. Esculin, 7. ONPG, 8. Gas, 9. Mannitol, 10. Motility, 11. Nitrate Broth, 12. Oxidase, 13. Catalase

Table.4 Evaluation of NPK activity of Cr(VI) resistant isolates

Bacteria	Nitrogen fixation	Phosphate solubilization (SI)	Potassium solubilization (SI)
Paenibacillus sp. **CTSI-01**	+	-	-
Staphylococcus sp. **CTSI-02**	+	-	-
Bacillus sp. **CTSI-03**	+	-	-
Bacillus sp. **CTSI-04**	+	-	-
Brevibacillus sp. **CTSI-05**	+	-	-
Micrococcus sp. **CTSI-06**	+	1	1
Bacillus sp. **CTSI-07**	+	-	-
Staphylococcus sp. **CTWI-01**	+	-	-
Bacillus sp. **CTWI-02**	+	-	-
Micrococcus sp. **CTWI-03**	+	-	1.2
Bacillus sp. **CTWI-04**	+	-	-
Brevibacillus sp. **CTWI-05**	+	-	-
Enterobacter sp. **CTWI-06**	+	0.57	1.3
Acinetobacter sp. **CTWI-07**	+	1	0.83

Evaluation of NPK activity of Cr(VI) resistant bacteria

Interestingly, amongst 14 chromium resistant bacterial isolates, three isolates such as *Micrococcus* sp. CTSI-06, *Enterobacter* sp. CTWI-06 and *Acinetobacter* sp. CTWI-07 depicted nitrogen fixation, phosphate and potassium solubilization capability as revealed from solubility index (Table 4). Moreover, several studies have reported utilization of chromium resistant and plant growth promoting rhizobacteria (PGPR) for the bioremediation of toxic metals (Samuel *et al.,* 2013; Sobariu *et al.,* 2016; Ndeddy and Babalola, 2016) to reclaim and restore soil properties.

In conclusion, the locally isolated strains showed higher tolerance or resistance to Cr (VI) as well as nitrogen fixation, phosphate and potassium solubilization capability. The advantages of selecting suitable native bacterial strains from Sukinda mining area for bioremediation purpose to minimize inhibitory effects of other co-contaminant present with chromium. Thus, these potential chromium resistant and NPK activity showing locally isolated bacterial strains may be used for detoxification of Cr(VI) as well as to increase nutrient availability of chromium contaminated soils. Further research is highly indispensible to study the hexavalent chromium reduction potentiality of the bacterial isolates.

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