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To cite this article: Avishek Dutta, Sayanti Ghosh, Jayanta D. Choudhury, Riddhi Mahansaria, Malancha Roy, Asish Kumar Ghosh, Tarit Roychowdhury & Joydeep Mukherjee (2017): Isolation of indigenous *Staphylococcus sciuri* from chromium-contaminated paddy field and its application for reduction of Cr(VI) in rice plants cultivated in pots, *Bioremediation Journal*, DOI: [10.1080/10889868.2017.1282935](https://doi.org/10.1080/10889868.2017.1282935)



To link to this article: <http://dx.doi.org/10.1080/10889868.2017.1282935>

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## Isolation of indigenous *Staphylococcus sciuri* from chromium-contaminated paddy field and its application for reduction of Cr(VI) in rice plants cultivated in pots

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### ABSTRACT

Accumulation of Cr(VI) in rice seeds cultivated in Cr-contaminated soil of the Sundarbans (India) is an environmental problem. Cr(VI) concentration in this soil was  $6.2 \pm 0.3$  mg/kg, whereas total chromium was  $32.04 \pm 1.60$  mg/kg. A Cr(VI)-removing bacterium isolated from Cr-contaminated paddy field soil of Sundarbans was identified as *Staphylococcus sciuri*. Enrichment culture of *S. sciuri* was applied to pot cultivation of rice in Cr-contaminated soil. After 8 weeks,  $71 \pm 3\%$  Cr(VI) (final concentration  $2.15 \pm 0.01$  mg/kg) and  $65 \pm 2\%$  total Cr removal (end concentration  $11.3 \pm 0.5$  mg/kg) were attained in bacterium-treated soils. Growth parameters indicated healthy development of plants cultivated in bacterium-treated soils that was not observed in control plants. Total Cr removal attained in rice seeds of plants cultivated in bacterium-treated soils compared with control rice seeds was  $78 \pm 4\%$ . Total Cr concentration in test seeds was  $0.72 \pm 0.05$  mg/kg (World Health Organization [WHO] permissible limit: 1.30 mg/kg), whereas the same in control seeds was  $3.27 \pm 0.16$  mg/kg. Cr(VI) reduction achieved in rice seeds cultivated in bacterium-treated soil compared with control rice seeds was  $95 \pm 5\%$ . Cr(VI) concentration in rice seeds cultivated in treated soil was  $0.050 \pm 0.003$  mg/kg, whereas the same in untreated control was  $0.93 \pm 0.05$  mg/kg. Successful paddy field soil bioremediation by any *Staphylococcus* species was demonstrated for the first time.

### KEYWORDS

Agriculture; biotransformation; 16S rRNA; Sundarbans; tannery

### Introduction

The Sundarbans, one of the world's largest single block of mangrove ecosystem and home to about 4 million people in India, is a unique example of coexistence of human and terrestrial life. Agriculture is the main occupation of the people in Sundarbans (Singh et al. 2010). Paddy is grown twice a year in the Indian Sundarbans. An unexpected problem emerged in 2009 when a consignment of rice for export to the European Union was rejected due to high chromium content in rice seeds. Previous environmental monitoring showed that Sundarbans sediments were enriched with chromium (Silva Filho et al. 2011). Cr(VI) present in chromium-contaminated tannery solid wastes is an important environmental concern owing to its extreme toxicity. This study was carried out in West Bengal State, which has been identified as one of the regions

where Indian tanneries are concentrated (Dhal et al. 2013). It is presumed that the chromium waste discharged into the Ganges-Hooghly river system is eventually deposited downstream in the soil of the Sundarbans located in the mouth of the Ganges.

Indigenous bacteria from contaminated environments are better adapted to the pertinent contaminants than introduced microbes, a reason in favor of selecting native bacteria for bioremediation (Panneerselvam et al. 2013). This logic has been reiterated for Cr because viable indigenous organisms develop some degree of resistance to Cr(VI) (Narayani and Shetty 2013) and they presumably possess mechanisms that allow them to remove this pollutant (González et al. 2014). Thus, the bioremediation approach is to transform Cr(VI) into less toxic and less mobile Cr(III) (Chai et al. 2009). Microbial reduction of Cr(VI) in

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soils and sediments contaminated by tannery wastes has been achieved by microbial reduction in presence of molasses, application of microbes derived from cow manure and sediments of sites accumulating tannery effluents, and bacterial leaching (Dhal et al. 2013). However, no investigation has been done on the reduction of Cr(VI) in contaminated agricultural soil followed by subsequent detection in the economic part of rice plant cultivated in the treated soil. The present objectives are (1) isolation of Cr(VI)-removing bacteria from paddy field soil of the Sundarbans; (2) identification of the most robust bacterium displaying Cr(VI) reduction activity; and (3) application of enrichment culture of the selected bacterium for rice cultivation in pots containing Cr(VI)-contaminated soil and assessment of bioremediation.

## Materials and methods

### Measurement of soil hexavalent Cr

Soil samples were collected from a paddy field of Bally Island, Sundarbans, India (latitude  $22^{\circ}6'47.87''$ , longitude  $88^{\circ}44'29.08''$ ) at the end of the monsoon season in 2013. Soil sample (2.5 g) was digested using 50 ml 0.28 M  $\text{Na}_2\text{CO}_3$ /0.5 M NaOH solution in presence of 0.5 ml of 1 M phosphate buffer and 400 mg of  $\text{MgCl}_2$  and heating at 90–95°C for 60 min. The soil digestate was cooled and filtered using 0.45- $\mu\text{m}$  filter paper, and the pH was adjusted to  $7.5 \pm 0.5$  using 5 M  $\text{HNO}_3$  (US Environmental Protection Agency [USEPA] method 3060A). Ninety-five milliliters of the extract was transferred to a 100-ml volumetric flask, and 2.0 ml diphenylcarbazide solution was added.  $\text{H}_2\text{SO}_4$  was added to the sample to obtain pH  $2 \pm 0.5$ , volume made up to 100 ml with water, kept for 5–10 min for color development, and finally absorbance measured at 540 nm (USEPA method 7196A).

### Measurement of soil total Cr

Soil sample (1.0 g) was taken in a crucible, placed in a preheated muffle furnace at 200–250°C for 30 min, and ashed for 4 h at 480°C (Hseu 2004). Sample was cooled, 2 ml of 5 M  $\text{HNO}_3$  added, and evaporated to dryness. Sample was then heated to 400°C for 15 min, cooled, and moistened with four drops of distilled water. Concentrated HCl (2 ml) was added to the sample in a test tube, evaporated to dryness,

5 ml of 2 M HCl added, and the tube swirled. The solution was filtered through Whatman No. 42 filter paper and <0.45- $\mu\text{m}$  Millipore filter paper and transferred quantitatively to a 25-ml volumetric flask. Determination was done in an atomic absorption spectrophotometer (model AAnalyst 200; PerkinElmer, Waltham, MA, USA) having hollow cathode lamp (10 mA). Data were acquired with Winlab 32 software (Biswas and Bhattacharjee 2014).

### Isolation of hexavalent chromium-resistant bacteria

Two soil samples were serially diluted and plated on nutrient agar containing 100 ppm  $\text{K}_2\text{Cr}_2\text{O}_7$ . Duplicate of each plate was made. Simultaneously, the dilutions of the soil samples were plated on nutrient agar medium without  $\text{K}_2\text{Cr}_2\text{O}_7$  to obtain normal microbial growth. After 24 h of incubation at 35°C,  $\text{K}_2\text{Cr}_2\text{O}_7$ -containing plates were compared with the ones without  $\text{K}_2\text{Cr}_2\text{O}_7$ .

### Screening of hexavalent chromium-removing bacteria

Hexavalent chromium-resistant bacteria were grown in Luria broth with 50 ppm Cr(VI). Blank samples without bacterial inoculant were prepared. After 24 h of incubation with shaking (100 rpm) at 35°C, the broth was centrifuged (10000 rpm for 6 min) and Cr(VI) in the supernatant was measured. Bacteria having more than 95% Cr(VI) reduction potential were further pursued for enhancement of Cr reduction.

### Enhancement of hexavalent chromium reduction

The selected bacteria were cultivated in (1) nutrient broth; (2) Luria broth; (3) Luria broth amended with 1% glucose; (4) chemically defined (CD) broth amended with 0.2% tryptone; and (5) CD broth amended with 0.2% tryptone and 1% glucose. All the media contained 50 ppm Cr as  $\text{K}_2\text{Cr}_2\text{O}_7$ . Blank samples without any bacterial inoculant were considered. Samples were incubated at 35°C with shaking at 100 rpm for 24 h. The broth was centrifuged (10,000 rpm for 6 min), and Cr(VI) in the supernatant was measured by USEPA method 7196A. Medium pH in all experiments was measured with a Eutech Instruments (Singapore) model pH 700 pH meter. All determinations were carried out thrice in duplicate sets.

### **Correlation between Cr(VI) tolerance and reduction**

Potent Cr(VI)-removing bacteria were grown in Luria broth amended with 1% glucose and different concentrations of Cr(VI) (50–400 ppm). Blank samples without bacterial inoculant were prepared. After 24 h of incubation (100 rpm at 30°C), the broth was centrifuged (10,000 rpm for 6 min), and Cr(VI) in the supernatant was measured. All determinations were carried out thrice in duplicate sets.

### **Identification of the Cr(VI)-removing bacterium selected for pot study**

The selected bacterium was cultivated in Luria broth amended with 1% glucose, its genomic DNA extracted, and the 16S rRNA gene amplified following Mahansaria, Choudhury, and Mukherjee (2015). The 16S rDNA sequence was analyzed in the National Center for Biotechnology Information (NCBI) server (<http://www.ncbi.nlm.nih.gov>) using the BLAST tool. The colony characteristics, microscopic features, and Gram reaction were recorded following standard protocols.

### **Rice cultivation in pots**

Rice seeds (*Oryza sativa*, variety *Dudheswar*, Boro paddy, partially photoinensitive, scented, small, and short grains) were collected from farmers of Bally Island. Seeds were soaked overnight in saline water, wrapped with paper, and kept for a day after which seeds germinated. Soil collected from the paddy field of Bally Island (on 23 July 2014) was taken into a pot (depth 20 cm), germinated rice seeds spread, covered with soil, and moistened. Seedlings developed after 1–2 days. Eight pots (four test and four control), each containing 2.5 kg Cr(VI)-contaminated soil collected from Bally Island, were planted with 10 cm seedlings (4 seedlings in each pot) on 3 August 2014. Soils were conditioned with a mixture of bone meal, blood meal, and organic compost at 4:2:1 N:P:K ratio. Pots were water logged (4 cm above soil) and placed in the open to provide the required temperature, sunlight, and rain water (Directorate of Rice Development, Patna, India 2002).

### **Soil treatment with chromium-removing bacterium**

Hexavalent chromium-removing bacterium was inoculated in 100 ml Luria broth amended with 1%

glucose, kept in an incubator (100 rpm) for 10 h at 30°C until OD<sub>600</sub> of 1.8–2.0 was reached. After 1 week of replantation, 90 ml of this culture was poured in each of the four test pots but not in the other four pots that were considered as controls. Residual Cr(VI) and total Cr concentrations in the soil were estimated after 8 weeks. Plant height, leaf length, leaf width, and number of leaves were recorded every week. Cow dung (2 g/kg) was added at the 10th week as fertilizer. After fruiting number of panicles, length of panicles, flag leaf length, and flag leaf width were registered. Summary of the events and measurements are provided as Supplementary Table 1.

### **Determination of total chromium in rice leaves and seeds**

After the 19th week, harvested rice leaf and seeds were dried at 65°C for 48 h. Leaves were cut into tiny pieces and the plant materials ground to dust in a mortar pestle. Approximately 2 g of sample was placed in a 50-ml digestion tube, and 3 ml concentrated HNO<sub>3</sub> (69%) and 2 ml H<sub>2</sub>O<sub>2</sub> were added to the sample. Samples were boiled on a hot plate at 95°C for 8 h until a clear solution was obtained. Concentrated HNO<sub>3</sub> was added at least thrice to the sample, and digestion continued until the volume was reduced to about 1 ml. After cooling, the sample volume was made up to 5 ml by using 1% HNO<sub>3</sub>. The solution was filtered through Whatman No. 1 filter paper, 0.2- $\mu$ m syringe filter, and transferred quantitatively to a 5-ml volumetric flask by adding distilled water. Total Cr was determined in an atomic absorption spectrophotometer (Hseu 2004; Zheljzkov and Nielson 1996).

### **Determination of Cr(VI) in rice leaves and seeds**

Approximately 0.4 g of sample (processed as described) was weighed and transferred into a 50-ml conical flask. Twenty milliliters of 0.1 M Na<sub>2</sub>CO<sub>3</sub> was added, and contents of the conical flask were boiled for 10 min. After cooling, the sample was centrifuged at 10000 rpm for 20 min. The supernatant was filtered through Whatman No. 1 filter paper and diluted to a final volume of 20 ml with distilled water (Panichev et al. 2005). Determination was done in an atomic absorption spectrophotometer.

## Results and discussion

### Chromium concentration in soil before treatment

The hexavalent chromium in *Sundarbans* soil was  $6.2 \pm 0.3$  mg/kg ( $n = 4$ ), whereas the total chromium in this soil was  $32.04 \pm 1.60$  mg/kg ( $n = 4$ ). This composition corroborates Bhattacharyya et al. (2005) who observed that under substantially reduced conditions of submerged tropical paddy fields, Cr solubility increases appreciably and Cr(III) constitutes the main fraction of the total soluble Cr. Similarly, Bagdon and Hazen (1991) estimated the ratio of hexavalent to total chromium of Cr-contaminated New Jersey soils (USA) as 0.14. The same ratio in our study appears as 0.19, indicating constitutional similarity amongst Cr-containing soils. According to Canadian Council of Ministers of the Environment (1999), the permissible level of total Cr in agricultural soil is 64 mg/kg and allowable level of Cr(VI) is 0.4 mg/kg. Evidently, *Sundarbans* soil exceeds this limit, justifying remedial measures.

### Isolation of Cr-resistant bacteria

Initially, Cr(VI) reduction potential of chromium-resistant bacteria was evaluated. Twenty-one Cr(VI)-resistant bacteria were isolated, of which 5 were capable of greater than 95% reduction of hexavalent chromium. Thus, Cr(VI) resistance and Cr(VI) reduction were unrelated properties, supporting the report of Bopp and Ehrlich (1988). Similar results were reported by Camargo et al. (2003), Qazilbash et al. (2006), and Okeke et al. (2008) who isolated several chromium-resistant bacteria from chromium-contaminated soils, but Cr(VI) reduction activity was detected in few isolates. Chromium-resistant bacteria have been previously isolated from different industrial discharges (Zahoor and Rehman 2009) and tannery effluent (Batool, Yrjälä, and Hasnain 2012). This is the first report on isolation of Cr(VI)-resistant bacteria from Cr-contaminated paddy field.

### Enhancement of Cr(VI) reduction

As the next step, Cr(VI) reduction was increased through development of suitable medium and cultivation conditions. In nutrient broth, no significant Cr(VI) reduction (2.65–9.69%,  $n = 30$ ) was observed for the five potent Cr(VI)-removing bacteria, although significant growth was observed for these bacteria.

Thus, Cr(VI) resistance and Cr(VI) reduction were unrelated. Cr(VI) reduction was significantly higher (90.32–97.12%,  $n = 30$ ) in Luria broth compared with nutrient broth. The presence of tryptone in Luria broth might act as an electron donor for Cr(VI) reduction (Ogg and Patel 2011). Similar result showing significant chromium reduction by *Enterobacter cloacae* HO1 in presence of tryptone in culture medium was reported by Ohtake, Fujii, and Toda (1990). Interestingly, higher Cr(VI) reduction (98.82–99.36%,  $n = 30$ ) with reduced incubation time (22–24 h) was observed in the potent bacteria when 1% glucose was added to Luria broth. Glucose enhances Cr(VI) reduction efficiency by acting as an extra electron donor for reduction of Cr(VI) (Garbisu et al. 1998). This observation was supported by Wang and Xiao (1995) and Ohtake, Fujii, and Toda (1990). It is possible that glucose increased microbial activity responsible for the reduction of Cr(VI). However, glucose addition to Luria broth produced mixed acid, thus lowering the pH and utilizing NADH (Cortassa et al. 2002; Lin, Bennett, and San 2005), which also acts as an electron donor for Cr(VI) reduction, thereby lowering the extent of Cr(VI) reduction (Wang and Shen 1995). To overcome this limitation, the CD medium was formulated containing  $\text{KH}_2\text{PO}_4$ ,  $\text{Na}_2\text{HPO}_4$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{CaCl}_2$ , and  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ . The first two ingredients prevented the pH drop by acting as a buffer system. This medium was amended with 0.2% tryptone as nitrogen source. Although this medium did not contain any carbon source, moderate Cr(VI) reduction ( $50 \pm 2\%$ ,  $n = 30$ ) was demonstrated. Intriguingly, there was remarkable improvement in Cr(VI) reduction (100% in 20 h) when 1% glucose was added to the CD medium. After 20 h of incubation, no pH change was observed. Similar increase in Cr(VI) reduction by adding glucose as an electron donor were reported by Bae et al. (2000). One isolate (S141), however, showed reasonable extent of Cr(VI) reduction in CD medium ( $70 \pm 2\%$ ,  $n = 6$ ) and very high ( $99 \pm 1\%$ ,  $n = 6$ ) in the complex (Luria broth with glucose) medium and was selected as the robust strain suitable for field application. The mechanism of bacterial reduction for Cr(VI) differs from strain to strain dependent upon their nutrient utilization patterns, which directly influences the resistance/tolerance to chromate (Dhal et al. 2013). Thereafter, a correlation between Cr(VI) tolerance level and reduction was evaluated.



### Cr(VI) tolerance and Cr(VI) reduction

High ( $99 \pm 1\%$ ,  $n = 30$ ) Cr(VI) reduction in all five Cr(VI)-removing bacteria was recorded at 50 ppm Cr(VI). Significantly low Cr(VI) reduction ( $25 \pm 5\%$ ,  $n = 30$ ) was noted at 100 and 200 ppm, although cell growth was adequate. Cell growth as well as Cr(VI) reduction was imperceptible at 300 and 400 ppm. Similar results were observed by Wang and Xiao (1995) where Cr(VI) reduction by *Bacillus* sp. decreased at higher concentrations. Increased Cr(VI) concentrations (300 and 400 ppm) damaged the bacterial cell due to its strong oxidizing potential (Kotaś and Stasicka 2000). Liu et al. (2006) reported similar effect on bacterial cells but at lower Cr(VI) concentrations. Next, it was considered essential to determine the identity of isolate S141, which was selected for field application.

### Taxonomic identification of isolate S141

The 16S rDNA sequence (GenBank KU377302) of the selected isolate (S141) showed 100% identity with *Staphylococcus sciuri* (Supplementary Table 2). The agar colonies were circular, gray-white, 10 mm in diameter, with moderately undulate edge. Microscopic observations depicted the bacterium as gram-positive coccoid, confirming the type strain description (Kloos, Schleifer, and Smith 1976). *S. sciuri* is a common bacterium found in a broad range of habitats such as animals, humans, and the environment (Nemeghaire et al. 2014). Chromium resistance has been confirmed in *Staphylococcus capitis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* (Narayani and Shetty 2013). However, successful Cr(VI) reduction and soil bioremediation by any species of *Staphylococcus* is currently being demonstrated for the first time.

### Assessment of soil bioremediation

We determined chromium concentrations in soil after bacterium treatment. Hexavalent Cr removal observed after 8 weeks in soils treated with *S. sciuri* was  $71 \pm 3\%$  ( $n = 4$ ), and the final Cr(VI) concentration in the soil was  $2.15 \pm 0.01$  mg/kg ( $n = 4$ ) (initial concentration  $7.4 \pm 0.2$  mg/kg;  $n = 4$ ). The total Cr recorded was  $11.3 \pm 0.5$  mg/kg ( $n = 4$ ), implying  $65 \pm 2\%$  ( $n = 4$ ) lowering (initial concentration  $32.1 \pm 1.52$  mg/kg;  $n = 4$ ). Control pots showed  $26 \pm 1\%$  ( $n = 4$ ) spontaneous Cr(VI) reduction where final Cr(VI)

concentration was  $5.01 \pm 0.20$  mg/kg ( $n = 4$ ) (initial concentration  $6.7 \pm 0.2$  mg/kg;  $n = 4$ ). The concentration of total Cr was  $27.4 \pm 0.7$  mg/kg ( $n = 4$ ), resulting in  $14.5 \pm 0.5\%$  ( $n = 4$ ) spontaneous decrease (initial concentration  $32.2 \pm 1.87$  mg/kg;  $n = 4$ ). We further observed growth of rice plants in bacterium-treated soils.

### Effect of soil bioremediation on rice plant growth parameters

Average plant height recorded for plants cultivated in treated soils was  $69 \pm 4$  cm ( $n = 16$ ), and the same parameter observed for control plants was  $58 \pm 3$  cm ( $n = 16$ ). Average plant leaf length was  $50.5 \pm 2.5$  cm ( $n = 16$ ) in plants treated with *S. sciuri* compared with average  $39 \pm 2$  cm ( $n = 16$ ) in the control plants. Average plant leaf width in plants cultivated in treated soils was  $1 \pm 0.2$  cm ( $n = 16$ ), whereas the same parameter recorded in plants grown in control soils was  $0.67 \pm 0.1$  cm ( $n = 16$ ). Average number of leaves in plants grown in treated soils was  $4 \pm 1$  ( $n = 16$ ), whereas the same parameter registered in plants cultivated in control soils was  $3 \pm 1$  ( $n = 16$ ). After fruiting, the average number of panicles observed in plants cultivated in treated soils was  $10 \pm 1$  ( $n = 16$ ), whereas the number of panicles in the control plants was  $3 \pm 1$  ( $n = 16$ ). The average length of panicles in plants cultivated in soils treated with *S. sciuri* was  $17.8 \pm 0.9$  cm ( $n = 16$ ), whereas the same parameter noted in control plants was  $10.4 \pm 0.5$  cm ( $n = 16$ ). The average length of flag leaves was  $22.7 \pm 1.2$  cm ( $n = 16$ ) in plants cultivated in bacterium-treated soils, whereas the same parameter recorded in control plants was  $11.5 \pm 0.6$  cm ( $n = 16$ ). The average width of flag leaves in plants grown in bacterium-treated soil was  $0.9 \pm 0.1$  cm ( $n = 16$ ), and the same parameter recorded in control plants was  $0.5 \pm 0.1$  cm ( $n = 16$ ). Thus, all parameters indicated better and faster growth in plants cultivated in bacterium-treated soils compared with control plants. This result can be justified as follows.

Application of *S. sciuri* lowered Cr(VI) level in the soil. So, lower amount of Cr(VI) was available to the plants cultivated in bacterium-treated soil and thus plant growth was not adversely affected. Conversely, Cr(VI) was available to the plants in the control pots, which resulted in hindered plant growth. Upon Cr exposure, leaf numbers per plant in wheat, leaf area,

and biomass of *Albizia lebbek* were reduced. Lowering of size, burning, and chlorosis of spinach leaves were noted in plants growing in Cr-contaminated soils (Shanker et al. 2005). Application of tannery effluent lowered leaf area and dry weight in *Oryza sativa*, *Acacia holosericea*, and *Leucaena leucocephala* (Shanker et al. 2005). Increasing doses of Cr caused significant reduction in root and shoot lengths, total leaf area, and yield of paddy plants (Sundaramoorthy et al. 2010). Lowering in plant heights was observed in oats, *Curcumas sativus*, *Lactuca sativa*, and *Panicum miliaceum* as an effect of soil Cr(VI) (Shanker et al. 2005). Wheat plant biomass reduced significantly after exposure to chromium (Subrahmanyam 2008). The consequences of Cr on the plant processes during early growth and development finally results in lowering of yield and total dry matter as an effect of reduced production, translocation, and partitioning of assimilates to the economic parts of the plant. The usual mechanism of selective inorganic nutrient uptake may be disrupted by oxidative damage, thus allowing greater quantities of Cr(VI) to passively enter the roots. Further translocation of Cr(VI) to shoot may cause oxidative damage to the photosynthetic and mitochondrial apparatus ultimately manifesting in poor plant growth. Contrastingly, Cr(III) is kinetically inert to ligand substitution and consequently can form substitution inert metalloprotein complexes in vivo, therefore significantly reducing its role in causing toxic symptoms (Shanker et al. 2005). We then investigated if Cr(VI) concentration was lowered in rice seeds.

#### **Effect of soil bioremediation on rice seeds and leaves**

Total Cr removal observed in rice seeds obtained from plants cultivated in bacterium-treated soils compared with the control rice seeds obtained from plants cultivated in untreated soil was  $78 \pm 4\%$  ( $n = 16$ ). The permissible limit of Cr for plants is 1.30 mg/kg, as recommended by the World Health Organization (WHO) (Nazir et al. 2015). Total Cr concentration in test seeds was  $0.72 \pm 0.05$  mg/kg ( $n = 16$ ), whereas the same parameter in control seeds was  $3.27 \pm 0.16$  mg/kg ( $n = 16$ ). Hexavalent Cr reduction attained in the rice seeds cultivated in bacterium-treated soil compared with the control rice seeds was  $95 \pm 5\%$  ( $n = 16$ ). The average Cr(VI) concentration in rice seeds cultivated in treated soil was  $0.050 \pm 0.003$  mg/kg ( $n = 16$ ), whereas the same parameter in

untreated controls was  $0.93 \pm 0.05$  mg/kg ( $n = 16$ ). Total Cr removal noted in the leaves of the test plants compared with the leaves of the control plants was  $35 \pm 2\%$  ( $n = 16$ ). The total Cr concentration in the leaves of the treated plants was  $1.99 \pm 0.10$  mg/kg ( $n = 16$ ), whereas the same parameter in control plants was  $3.07 \pm 0.15$  mg/kg ( $n = 16$ ). The accumulation of Cr(VI) in the leaves of the plants grown in bacterium-treated soils was lower by  $89 \pm 2\%$  ( $n = 16$ ) compared with the control plants. Cr(VI) concentration in the economic part of the plant (seeds) was  $0.050 \pm 0.003$  mg/kg ( $n = 16$ ), which is within the safe limit as prescribed by WHO (Nazir et al. 2015). Our results showed lower accumulation of both total and Cr(VI) in leaves and rice seeds of plants grown in treated soil compared with the control plants. Similarly, lower amount of total and Cr(VI) accumulation was found in the straws and grains of paddy grown using low Cr-containing cow dung manure than that in plants grown using higher Cr-containing municipal solid waste compost (Bhattacharyya et al. 2005). *Pseudomonas aeruginosa* Rb-1 and *Ochrobactrum intermedium* Rb-2 when augmented in Cr(VI)-contaminated soil enhanced seed germination, shoot and root lengths, and numbers of roots of wheat plants growing in Cr(VI)-containing soils (Batool, Yrjälä, and Hasnain 2015). In this study, comparatively larger amounts of total and Cr(VI) were recovered in plant leaves than seeds. Total Cr accumulation in grains was low in comparison with roots, stems, and leaves of rice plants cultivated in Cr-contaminated soil of a chromite mining area (Mohanty et al. 2011).

#### **Conclusions**

Through this investigation, an attempt was made to solve a severe problem being faced by the farmers of the *Sundarbans*. An indigenous highly potent Cr(VI)-removing bacterium, *Staphylococcus sciuri*, from Cr-contaminated paddy field was isolated, and Cr(VI) reduction was demonstrated for the first time in the genus *Staphylococcus*. Successful bioremediation in the pot scale was demonstrated, as evident from minimal contents of Cr recovered in the economic part of the rice plant. Future studies will concentrate on speciation of Cr(VI) and Cr (III), elucidation of the mechanistic pathways of Cr(VI) reduction in *Staphylococcus sciuri*, and translation of this pilot study to the agricultural field.

## Acknowledgment

The authors thank Anil Mistri and Subhash Mondal for logistic support during field work.

## Funding

Financial support from Jadavpur University is gratefully acknowledged.

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