

Research Article

Isolation of Alkaliphilic Bacterium *Citricoccus alkalitolerans* CSB1 : An Efficient Biosorbent for Bioremediation of Tannery Waste Water

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Abstract

The present study designed to isolate and characterize an alkaliphilic chromate-resistant bacterium from crude *Citricoccus alkalitolerans* CSB1 by 16S rDNA sequence analysis. The isolate could tolerate 10^{-1} of hexavalent chromium. Removal of Cr (VI) at concentration 10^{-1} after 60 hours was found 84.6%. The results on EDX analysis demonstrated surface binding as well as intracellular uptake of chromium by the bacterial cell. Zeta potential measurement indicates that the cell surfaces display a net negative charge at pH 10.0 (-29.80 mV). This was supported by Fourier transform infrared spectroscopy analyses demonstrating that the cells are dominated by surface proton releasing ligands, including carboxyl, phosphoryl and amino functional groups. The negative zeta potential which might be facilitating Cr binding. Cr adsorption experiments further reveal that functional groups play a primary role in metal adsorption. Furthermore, the Cr (VI) sorption by 1 fresh biomass. Results demonstrated that the *C. alkalitolerans*

Keywords: Alkaliphiles; *C. alkalitolerans* CSB1; FTIR; Zeta potential; SEM- EDX; Tannery effluent

Introduction

India is the third largest leather producer in the world, behind China and Italy. The states of Tamil Nadu, West Bengal, and Uttar Pradesh together have 88% of the tannery units of the country. The river Ganga in Kanpur (India) is reportedly one of the most polluted places and is heavily contaminated with heavy metals. The nature and behavior of Cr in wastewater depends on the physicochemical conditions of the effluents originating from various industrial sources [1-10]. The tanning industry is highly water intensive industry and generates a huge quantity of wastewater which is characterized by high organic load, suspended solids, high salinity (1% to 10% NaCl by wt.) and presence of excess quantity of Cr (VI) which hinder the treatment of tannery waste water [11-19]. Although Cr (III) is an essential micronutrient, soluble Cr (VI) but it is as carcinogen and is found to be toxic to all living beings [20-33]. For removal of such toxic metals by the different Physico-chemical methods of waste water treatment includes reverse osmosis, solvent extraction, lime coagulation, ion exchange, chemical precipitation, and membrane separation process, filtration, and incineration are being used [22,34-40], but these processes are cost effective and require large area to dispose of the tannery sludge. Various microorganisms capable of reducing the level of toxic pollutants can be used for biological treatment of waste water which can minimize the recurring expenses, high energy requirement and generation of secondary sludge [41-69]. Detoxification of hexavalent chromium has been carried out by using variety of bacteria under both aerobic and anaerobic conditions e.g. *Pseudomonas fluorescens* LB 300 [70-76], *Enterobacter cloacae* HO1 [77], *Bacillus* sp. [78]. The ability of the cell wall of *Bacillus subtilis* to interact with different heavy metals has been much studied [71]. Several reports have shown that the cell walls of Gram-positive cocci such as those of *Staphylococcus xylosum* and *Micrococcus luteus* have an affinity for metal ions [50], Application of indigenous microbes with greater tolerance against high concentrations of heavy metals and may play an important role in detoxifying the contaminated water and soil. For example, Wei et al. [79-82] recently identified *Agrobacterium* (CCNWR33-2) from the Taibai gold mining region in China as the

bacterial strain exhibiting resistance against heavy metals and was able to grow in the presence of 2 mM of copper and lead. Various workers have reported that the chromate reduction by bacteria is restricted to acidic and near-neutral pH conditions [64]. Very few reports have described chromate detoxifying bacteria from alkaline conditions [15,72]. Since, chromium containing tannery effluents are haloalkaline solutions with a high organic loading, there is need to search for the biological agents exhibiting tolerance against salinity, chromium and alkaline pH conditions which contribute to characteristic feature of tannery waste water [56]. The chromium tolerant microbes adapted to both hypersaline and extreme alkaline pH conditions [83] can be useful to overcome the challenges faced by the tannery water treatment plants. [11]. The major aim of this study was to isolate and characterize the alkalitolerant and chromium resistant bacterium and assess its potential to remove the pollutants load in tannery waste water.

Materials and Methods

Isolation and Identification: The bacterium was isolated from Sambhar Salt Lake, Rajasthan (India). The bacterium was allowed to grow in CM media used for halophiles. The medium containing Yeast extract-10, KCl -2, FeCl₃ -0.02, Casamino acid -7.5, tri-sodium citrate-3.0, MgSO₄·7H₂O-20, NaCl -100 (g L⁻¹) and supplemented with K₂Cr₂O₇ -20 µg mL⁻¹ at pH

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8 ± 0.02 theasks were incubated at room temperature for 24 h on an orbital shaker. After 24 h, the bacterial strain was isolated from salt containing chromium enriched agar plate. The bacterium CSB1 was isolated and identification was done by 16S rRNA sequencing as described elsewhere [11].

16S rRNA Sequencing and Phylogenetic Analysis

The isolation of the CSB1 isolate, characterization and identification was done by biochemical tests and 16S rRNA sequencing as described in previous study [11]. The isolate was identified by 16S rDNA sequencing using Universal primers (forward primer; 27 F 5'-AGA GTTT GAT CMT GGC TCAG -3' and reverse primer with modification; 1492 R 5'-TA CCG YTA CCT TGT TAC GAC TT-3'). Multiple alignments with sequences of actinobacteria of the family Micrococcaceae and calculations of levels of sequence similarity were carried out using CLUSTAL_X [70]. A phylogenetic tree was reconstructed using the neighbour-joining method [58]. Later, nucleotide sequence data were deposited in the Gen-Bank sequence database. The online program BLASTn was used to find out the related sequences with known taxonomic information in the databank at NCBI website (<http://www.ncbi.nlm.nih.gov/BLAST>) to accurately identify the strain CSB1.

Sample Collection and Bioremediation

The sample tannery effluent (waste water) was collected from Super House Tannery No-1 District Unnao (Uttar Pradesh) India, in the sterile glass bottles. Immediately, after collection of tot

A. Adsorption of Cr(VI) by *Citricoccus alkalitolerans*

Bacterial culture after 24 hour growth in CM broth were harvested and dried in hot air oven at 40°C overnight. The adsorption of Cr (VI) at different concentrations of chromium 15 -75 µg mL⁻¹ was measured using 1 mg dry biomass mL⁻¹ as adsorbent. The samples were withdrawn at regular intervals of 15 min up to 60 min. The cell suspension was centrifuged and the pellets were washed with (EDTA) 5 mM solution and centrifuged (8000 rpm, 10 min.). The EDTA washed fraction was used for measuring the centrifuged adsorbed chromium. Intracellular Cr (VI) concentrations was determined in the EDTA washed pellets after digesting it with 10-15 mL HNO₃ and HClO₄ (ratio of 3:1) on hot plate at 60°C to 80°C until mixture became colorless. The solution was filtered through Whatman No. 42 filter paper before analysis. The chromium concentrations was determined by Atomic absorption spectrophotometer (AA 240 FS: Fast Sequential AAS Varian, Netherland) at a wavelength 357.87 nm. The uptake rate was expressed as µg Cr (VI) mg⁻¹ dry weight of biomass.

$$\text{Metal concentration mg / L} = \frac{A - B}{C} \times D$$

hydroxyl and secondary amide shifted to 3295.8 cm^{-1} due to the mainly N-H stretching in the sec-amide proteins indicating the involvement of membrane proteins in chromium binding. The IR peak 2927.0 cm^{-1} depicted the lipids, proteins and polysaccharides, the shifting of these bands to 2931.4 indicated the involvement of N-H and C-H asymmetric stretching of phospholipids and polysaccharides in the adsorption process. A most prominent feature of the metal treated cells was the appearance of a relatively strong and well-resolved peak of C=O group or esters groups stretching vibration, at 1735.7 cm^{-1} was due to changes in lipids after chromium treatment. The wavenumber of 1652.1 cm^{-1} of amide-I band of proteins indicating asymmetric stretching vibrations C=O group proteins, the peak corresponding of protonated carboxyl groups. And the deprotonated carboxylate anion, COO^- , was expected to appear around 1542 cm^{-1} in alkaline cell suspensions. It

was obscured by the intense amide bands in the range of $1500\text{-}1650\text{ cm}^{-1}$. However, the symmetric stretching of COO^- vibration appeared around 1396 cm^{-1} . A characteristic peak of -COOH group of side chains of Amino and Fatty acids observed around 1401.0 cm^{-1} in untreated cells shifted to lower wavenumbers 1386.9 cm^{-1} due to interaction of

using the Helmholtz-Von Smoluchowski for selected isolate in 10 mM

The total hardness of tannery effluent after bacterial treatment was reduced by more than 50% as compared to control (untreated).

D

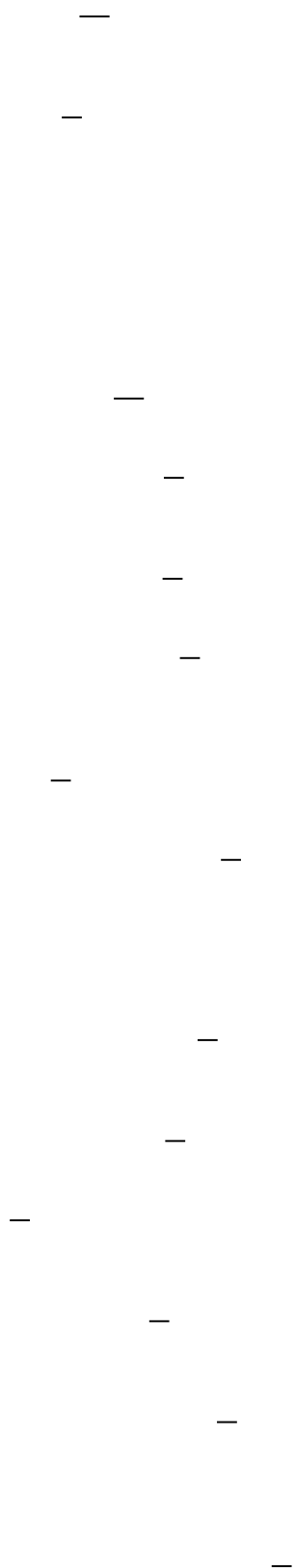
adsorption isotherm model was $15.8 \mu\text{g mg}^{-1}$ at room temperature and it found close to experimental value. The Langmuir equation was applied for monolayer sorption onto a surface with a finite number of identical sites which are homogeneously distributed over the adsorbent surface [39]. Further, analysis of the R^2 value (0.998) suggested that the equilibrium adsorption data fitted well with Langmuir isotherm model.

The Freundlich (KF and n) constants were calculated from the slope and intercept of the plot of $\log q_e$ vs. $\log C_e$ (Figure 11) and presented in Table 4 along with their correlation coefficient R^2 . The Kf value was found to be 0.323 (Table 2), indicated that adsorption coefficient for Cr binding on to CSB2 cell surfaces. The value of constant n was found to be 1.25, indicated beneficial adsorption process [6]. The value of constant n represented bonding strength between the Cr and biosorbent and n value between 1 to 10 indicated favorable adsorption process [6]. Comparatively low value of correlation coefficient R^2 (0.952) suggested that Freundlich adsorption isotherm model did not fit well to the experimental data for the biosorption of Cr (VI) onto biomass of CSB1.

P

Results of the analysis of the physicochemical parameters of untreated tannery effluent are depicted in Table 4. The results revealed that color of the untreated industrial effluent were blackish with unpleasant odor. This color may be contributed by undecomposed organic and inorganic matter. The Chromium concentration was about $114.2 \text{ (mg L}^{-1}\text{)}$. This was 56 times higher than recommended by CPCB (India) for irrigation water. There was a significant change in different parameters like hardness, chloride, BOD, COD etc. The isolates showed more than 80% reduction in BOD, 70% reduction in COD and more than 95.2% remediation of total chromium was observed in the batch cultures inoculated *C. alkalitolerans* CSB 1 after 96 hour of incubation.

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