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 $^{-1}$ of hexavalent chromium. Removal of Cr (VI) at concentration $^{-1}$ 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*

**K , :** Alkaliphiles; *C. alkalitolerans* CSB1; FTIR; Zeta potential; SEM- EDX; Tannery e uent

## I ,

India is the third largest leather producer in the world, behind China and Italy. e states of Tamil Nadu, West Bengal, and Uttar Pradesh together have 88% of the tannery units of the country. e river Ganga in Kanpur (India) is reportedly one of the most polluted places and is heavily contaminated with heavy metals. e nature and behavior of Cr in wastewater depends on the physicochemical conditions of the e uents originating from various industrial sources [1-10]. e 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 me-tals by the di erent Physico-chemical methods of waste water treatment includes reverse osmosis, solvent extraction, lime coagulation, ion exchange, chemical precipitation, and membrane separation process, ltration, and incineration are being used [22,34-40], but these processes are cost e ective and require large area to dispose o 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]. Detoxi cation of hexavalent chromium has been carried out by using variety of bacteria under both aerobic and anaerobic conditions e.g. *Pseudomonas uorescens* LB 300 [70-76], Enterobacter cloacae HO1 [77], *Bacillus* sp. [78]. e ability of the cell wall of *Bacillus* subtilis to interact with di erent heavy metals has been much studied [71]. Several reports have shown that the cell walls of Gram-positive cocci such as those of *Staphylococcus xylosus* and *Micrococcus luteus* have an a nity 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 identi ed Agrobacterium (CCNWRS33-2) 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 e uents 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]. e 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]. e 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.

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e bacterium was isolated from Sambhar Salt Lake, Rajasthan (India). e bacterium was allowed to grow in CM media used for halophiles. e medium containing Yeast extract-10, KCl -2, $FeCl_3$ -0.02, Casamino acid -7.5, tri-sodium citrate-3.0, $MgSO_4.7H_2O$-20, NaCl -100 (g $L^{-1}$) and supplemented with $K_2Cr_2O_7$ -20 µg $mL^{-1}$ at pH

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

### 16 , NA

e isolation of the CSB1 isolate, characterization and identi cation was done by biochemical tests and 16S rRNA sequencing as described in previous study [11]. e isolate was identi ed by 16S r DNA sequencing using Universal primers (forward primer; 27 F 5'- AGA GTTT GAT CMT GGC TCAG -3' and reverse primer with modi cation; 1492 R 5'- TA CGG 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. e online program BLASTn was used to nd 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.

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

**A , ' C, ( I) , ' ',**

Bacterial culture a er 24 hour growth in CM broth were harvested and dried in hot air oven at 40ºC overnight. e adsorption of Cr (VI) at di erent concentrations of chromium 15 -75 µg $mL^{-1}$ was measured using 1 mg dry biomass $mL^{-1}$ as adsorbent. e samples were withdrawn at regular intervals of 15 min up to 60 min. e cell suspension was centrifuged and the pellets were washed with (EDTA) 5 mM solution and centrifuged (8000 rpm, 10 min.). e EDTA washed fraction was used for measuring the centrifuged adsorbed chromium. Intracellular Cr (VI) concentrations was determined in the EDTA washed pellets a er digesting it with 10-15 mL $HNO_3$ and $HClO_4$ (ratio of 3:1) on hot plate at 60°C to 80°C until mixture became colorless. e solution was ltered through Whatman No. 42 lter paper before analysis. e chromium concentrations was determined by Atomic absorption spectrophotometer (AA 240 FS: Fast Sequential AAS Varian, Netherland) at a wavelength 357.87 nm. e uptake rate was expressed as µg Cr (VI) $mg^{-1}$ dry weight of biomass.

$$\textit{Metal concentration mg } L \quad \frac{A \;\; B \;\; C}{D}$$

hydroxyl and secondary amide shi ed 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. e IR peak 2927.0 $cm^{-1}$ depicted the lipids, proteins and polysaccharides, the shi ing 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 a er chromium treatment. e 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 1500-1650 $cm^{-1}$. 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 shi ed to lower wavenumbers 1386.9 $cm^{-1}$ due to interaction of

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

e total hardness of tannery e uent a er bacterial treatment was reduced by more than 50% as compared to control (untreated).

**D**

adsorption isotherm model was 15.8 µg $mg^{-1}$ at room temperature and it found close to experimental value. e Langmuir equation was applied for monolayer sorption onto a surface with a nite number of identical sites which are homogeneously distributed over the adsorbent surface [39]. Further, analysis of the R2 value (0.998) suggested that the equilibrium adsorption data tted well with Langmuir isotherm model.

e Freundlich (KF and n) constants were calculated from the slope and intercept of the plot of log qe vs. log Ce (Figure 11) and presented in Table 4 along with their correlation coe cient R2. e Kf value was found to be 0.323 (Table 2), indicated that adsorption coe cient for Cr binding on to CSB2 cell surfaces. e value of constant n was found to be 1.25, indicated bene cial adsorption process [6]. e 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 coe cient R2 (0.952) suggested that Freundlich adsorption isotherm model did not t well to the experimental data for the biosorption of Cr (VI) onto biomass of CSB1.

**P P A**

Results of the analysis of the physicochemical parameters of untreated tannery e uent are depicted in Table 4. e results revealed that color of the untreated industrial e uent were blackish with unpleasant odor. is color may be contributed by undecomposed organic and inorganic matter. e Chromium concentration was about 114.2 (mg $L^{-1}$). is was 56 times higher than recommended by CPCB (India) for irrigation water. ere was a signi cant change in di erent parameters like hardness, chloride, BOD, COD etc. e 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 a er 96 hour of incubation.

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