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Hexavalent chromium reduction potential of *Cellulosimicrobium* sp. isolated from common effluent treatment plant of tannery industries

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ABSTRACT

Present study deals with the isolation and characterization of a bacterium capable for the effective reduction of Cr(VI) from tannery wastewater. Based on the 16S rRNA gene sequence analysis, this bacterium was identified as *Cellulosimicrobium* sp. (KX710177). During the Cr(VI) reduction experiment performed at 50, 100, 200,and 300 mg/L of Cr(VI) concentrations, the bacterium showed 99.33% and 96.98% reduction at 50 and 100 mg/L at 24 and 96 h, respectively. However, at 200 and 300 mg/L concentration of Cr(VI), only 84.62% and 62.28% reduction was achieved after 96 h, respectively. The SEM analysis revealed that bacterial cells exposed to Cr(VI) showed increased cell size in comparison to unexposed cells, which might be due to either the precipitation or adsorption of reduced Cr(III) on bacterial cells. Further, the Energy Dispersive X-ray (EDX) analysis showed some chromium peaks for cells exposed to Cr(VI), which might be either due to the presence of precipitated reduced Cr (III) on cells or complexation of Cr(III) with cell surface molecules. The bacterium also showed resistance and sensitivity against the tested antibiotics with a wide range of MIC values ranging from 250 to 800 mg/L for different heavy metals. Thus, this multi-drug and multi-metal resistant bacterium can be used as a potential agent for the effective bioremediation of metal contaminated sites.

1. Introduction

The contamination of environments (soil and water) with various toxic metals is a serious threat for ecosystem and human health, and requires the implementation of appropriate remedial measures. Heavy metals, such as chromium, cadmium, mercury, arsenic, lead etc. are considered as major environmental pollutants due to their toxic effects on environment as well as on human health (Ray and Ray, 2009). In developing countries, different types of industrial wastes (solid and liquid) containing a number of toxic metals in high concentration are directly or indirectly discharged into the environment without adequate treatment (Dixit et al., 2015; Chandra et al., 2009). Industries such as metallurgical, chemical, refractory brick, leather, wood preservation, pigments and dyes are the major sources of toxic metals contamination in environment (USEPA, 1998; Ryan et al., 2002).

However, tannery industries are the major source of chromium contamination into the environment. Tannery industries consume a huge volume of water in tanning of hides and skin, as it is wholly a wet process and generate ~ 30–35 L of wastewater per kg skin/hides processed (Nandy et al., 1999). There are ~ 3000 tanneries in India, mainly located in the states of Tamil Nadu, West Bengal, Uttar Pradesh, Andhra Pradesh, Bihar, Gujarat, and Maharashtra, generating total ~ 1,75,000 m^3 wastewater per day (Kaul et al., 2005). In Uttar Pradesh, ~ 444 tanneries are in operation mainly in Kanpur and Unnao region generating 22.1 MLD of wastewater per day (CPCB, 2013) and this wastewater is reported to contain 0.01–4.24 mg/L of Cr(VI) (MOWR, 2013). However, most of the tanneries (nearly 80%) are engaged in chrome tanning process that releases ~ 2000–3200 t of Cr into the environment annually (Belay, 2010). The Cr concentration in tannery wastewater ranges between 2000 and 5000 mg/L, which is much higher than the permissible limit of 2 mg/L for wastewater discharge (Belay, 2010).

Like organic pollutants, metals are not degraded and tend to accumulate into the environment, may enter the food chain and cause toxic, genotoxic, mutagenic and carcinogenic effects (Chandra et al., 2011). Chromium compounds are well known to have toxic, genotoxic, mutagenic, and carcinogenic effects on humans, animals, plants, and as well as in microbes (Cheung and Gu, 2007; Mishra and Bharagava, 2016). In nature, chromium exists in several oxidation states ranging from − 2 to + 6, but only trivalent (III) and hexavalent (VI) forms of chromium is most prevalent and stable. Out of these two forms, hexavalent chromium [Cr(VI)] is highly toxic, mutagenic, teratogenic,

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carcinogenic to human and animals and has been designated as priority pollutant by US Environmental Protection Agency (USEPA) (1998). If Cr(VI) concentration into the environment exceeds > 0.05 mg/L, then it may affect the human physiology and if enter the food chain, it may cause severe health hazards such as skin irritation, nasal irritation, ulceration, eardrum perforation, and lung carcinoma etc. (WHO, 2011; Srinath et al., 2002).

Cr(VI) also acts as a strong oxidizing agent and exists only in oxygenated forms as hydro-chromate ($HCrO_4^-$), chromate (CrO_4^-) and dichromate ($Cr_2O_7^{-2}$) ionic species in aqueous systems. Cr(VI) compounds are comparatively more toxic than Cr(III) compounds due to their higher solubility in water, rapid permeability through biological membranes and subsequent interaction with intracellular proteins and nucleic acids (Thacker et al., 2006; Cheung and Gu, 2007). Although a number of conventional/traditional methods are reported either for removal or detoxification of Cr(VI) from industrial wastes such as chemical precipitation, reverse osmosis, ion-exchange, filtration, membrane technologies, evaporation recovery, absorption on coal, activated carbon, alum, kaolinite, and fly ash etc. (Saxena et al., 2016; Ahluwalia and Goyal, 2007). These methods are very costly, less effective and also generate a metal rich sludge as secondary pollutants. Therefore, it becomes very essential to develop an eco-friendly, cost-competitive and effective method for removal/detoxification of Cr(VI) for the safety of environment and human health protection.

However, microbial reduction of toxic Cr(VI) to non-toxic Cr(III) by chromium resistant bacteria (CRB) is the most pragmatic approach that offers an economical as well as eco-friendly option for chromate detoxification and bioremediation. Microbes have diverse resistance mechanisms to cope with chromate toxicity that enable them to survive in such harsh environmental conditions (Cervantes and Campos-Gracia, 2007). These detoxification strategies include biosorption, bioaccumulation and biotransformation by enzymatic reduction, diminished intracellular accumulation through either direct obstruction of ion uptake system or active chromate efflux, precipitation, and reduction of Cr(VI) to less toxic and less mobile Cr(III) (Cheung and Gu, 2003; Ramirez-Diaz et al., 2008). Hence, the objectives of this study were to isolate and characterize chromium resistant bacteria, which should be capable to reduce/detoxify the toxic Cr(VI) into less toxic and less mobile Cr(III) for environmental cleanup and human health safety.

2. Materials and methods

2.1. Collection of tannery wastewater

The tannery wastewater was collected from Common Effluent Treatment Plant (CETP) of Jajmau Unit, Kanpur (26°26'59.7228"N and 80°19'54.7335"E), Uttar Pradesh, India in a pre-sterilized conical flask (Cap. 2 L), brought to laboratory, maintained at 4 °C and used in analysis of physico-chemical parameters as well as for the isolation of bacterial strains capable for the reduction of hexavalent chromium.

2.2. Physico-chemical analysis of tannery wastewater

The physico-chemical analysis of tannery wastewater was made in triplicate as per the standard methods for the examination of water and wastewaters (APHA, 2012). The collected tannery wastewater was analyzed for pH, conductivity, BOD (5 days method), COD (open reflux method), total solids (TS), total dissolve solids (TDS) and total suspended solids (TSS) (drying method), Total nitrogen (TN) (Kjeldhal method) and Chloride ($AgNO_3$ titration method). Phosphate and sulphate was measured (Vanadomolybdo-phosphoric acid) colourimetric and ($BaCl_2$ precipitation) methods, respectively (APHA, 2012).

2.2.1. Analysis of heavy metals in tannery wastewater

The concentration of heavy metals (Cr, Zn, Mn Ni, Cd, and Fe) in collected tannery wastewater was determined by the acid digestion method and Atomic Absorption Spectrophotometer (AAS) (VARIAN AS240FS, Australia), following the standard methods for the examination of water and wastewater (APHA, 2012). The digestion of wastewater sample was performed by taking 100 mL filter sterilized wastewater sample in a conical flask containing 6 mL of conc. Nitric acid and 1 mL of perchloric acid (6:1). This mixture was swirled gently covered with watch glass and heated on hot plate at room temperature. The sample was digested on hot plate until yellow fumes were released and the solution become clear. After cooling, the acid solution was filtered by Whattman's filter paper No. 44 and the volume of samples was make up 10 mL by using deionized water and used for metal analysis with their respective standard metal solutions.

2.3. Isolation of chromium resistant bacterial strain and growth conditions

To isolate chromium resistant bacteria, the collected tannery wastewater was serially diluted and spreaded on Luria-Bertani (LB) agar plates amended with potassium dichromate (100 mg/L) and incubated at 37 °C for 24–48 h (Farag and Zaki, 2010). The morphologically distinct colonies appeared on potassium dichromate amended LB agar plates were screened for maximum chromium tolerance potential by subsequent transferring/sub-culturing on LB agar plates amended with increasing concentration (200–1000 mg/L) of potassium dichromate. Out of ten bacterial isolates, only one bacterium (SCRB10) was found capable to tolerate 800 mg/L concentration of Cr(VI) and selected for biochemical characterization, identification by 16S rRNA gene sequencing analysis and other studies.

2.4. Characterization and identification of bacterial isolate

2.4.1. Morphological and biochemical characterization

The isolated bacterium was characterized morphologically and biochemically following the standard protocols of Cowan and Steel's manual for the identification of medical bacteria (Barrow and Feltham, 1993) and identified based on 16S rRNA gene sequencing analysis.

2.4.2. 16S rRNA gene sequencing analysis and gene-bank accession number

The genomic DNA was prepared from overnight grown bacterial culture following the alkaline lysis method described by Kapley et al. (2001). About 5 µL DNA was used to amplify 16S rDNA gene using universal eubacterial primers (27F) 50-AGAGTTTGATCMTGGCTCAG-30 and (1492R) 50-TACGGYTACCTTGTTACGACTT-30 (Narde et al., 2004) and a 1500 bp product was amplified. The reaction mixture contained 5 µL template, 1X PCR buffer, 200 µM of each dNTP, 3.0 mM $MgCl_2$, 25 pmol of primer, and 2.5 units of Amplitaq DNA polymerase (Perkin Elmer) in a final reaction volume of 50 µL (Bharagava et al., 2009).

The thermocycling reactions were carried out by using Veriti® 96-well Thermal Cycler (Applied Biosystems, USA). The 16S rDNA fragment was amplified by 35-cycles, PCR initial denaturation at 95°C for 5min, subsequent denaturation at 94°C for 30s, annealing temperature at 50°C for 30s, extension temperature 72°C for 1.30min and final extension at 72°C for 7min). The PCR product was analyzed on 1% agarose gel and purified by using gel extraction kit (Merk Biosciences, Bangalore). The gel purified PCR products were made sequenced by Chromous Biotech, Pvt Ltd. (Bangalore, India) on ABI 3500 Genetic Analyzer, using Big Dye Terminator Version 3.1″. The partial sequences obtained were subjected to BLAST analysis using the online option available at www.ncbi.nlm.nih.gov/BLAST (Altschul et al., 1997) suggesting the identity of isolated bacterium. The phylogenetic tree was constructed by neighbour-joining method using NCBI database online phylogenetic tree builder (http://www.ncbi.nlm.nih.gov). Further, the sequences were also deposited to Gene-Bank under the accession no. KX710177.