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Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint

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Abstract Toxic, mutagenic and carcinogenic heavy metals from tannery industries cause the pollution of agricultural environment and natural water sources. This study aims to isolate, investigate and identify naturally occurring bacteria capable of reducing and detoxifying heavy metals (Chromium, Cadmium and Lead) from tannery effluent. Three isolates were identified up to genus level based on their morphological, cultural, physiological and biochemical characteristics as *Gemella* sp., *Micrococcus* sp. and *Hafnia* sp. Among them *Gemella* sp. and *Micrococcus* sp. showed resistance to Lead (Pb), chromium (Cr) and cadmium (Cd), where *Hafnia* sp. showed sensitivity to cadmium (Cd). All isolates showed different MICs against the above heavy metals at different levels. Degrading potentiality was assessed using Atomic Absorption Spectrophotometer where *Gemella* sp. and *Micrococcus* sp. showed 55.16 ± 0.06% and 36.55 ± 0.01% reduction of Pb respectively. On the other hand, moderate degradation of Cd was shown by *Gemella* sp. (50.99 ± 0.01%) and *Micrococcus* sp. (38.64 ± 0.06%). Heavy metals degradation capacity of *Gemella* sp. and *Micrococcus* sp. might be plasmid mediated, which might be used for plasmid transformation to transfer heavy metal accumulation capability. Therefore, identification of three bacteria for their heavy metal resistance and biodegradation capacity might be a base study to develop the production of potential local bioremediation agents in toxic tannery effluent treatment technology.

Introduction

Air, water and land which are the essential elements of life are contaminated constantly due to increasing population, rapid urbanization and industrialization (Chhikara and Dhankhar,

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2008). At present, the bioaccumulation of heavy metals in environment is a major warning to human life (Yigit and Ahmet, 2006; Hooda, 2007). Water pollution caused by industrial wastage, is frequent (Ogedengbe and Akinbile, 2004) by toxic sludge, heavy metals, and solvents as they fall into natural water sources and agricultural environment.

Heavy metals containing industrial effluent cause health hazards to plants, animals, aquatic life and humans increasing pressures on the flora and fauna (Robin et al., 2012). Among industrial usage of heavy metals, tannery industries use a significant part of it. Tannery effluent is highly polluted because it contains imbalance suspended solids, nitrogen, conductivity, sulfate, sulfide and chromium, copper, cadmium and manganese, biological oxygen demand (BOD) and chemical oxygen demand (COD) (Mondal et al., 2005; Zahid et al., 2006). In Bangladesh unprocessed tannery effluents are released into water sources (Favazzi, 2002; Verheijen et al., 1996). Consequently, the elevated concentrations of some heavy metals are found in agricultural soils located in surrounding areas to the tannery industries which exceed the tolerable limit.

Lead and cadmium which are major contaminants found in the environment, are extremely poisonous to human(s), animals, plants and microbes which can damage cell membranes, alter particularity of enzymes, and destroy the structure of DNA. This toxicity is created by the displacement of essential metals from their native binding sites or ligand interactions (Olaniran et al., 2013).

Chrome powder and chrome liquor are applied in tanning industry, and are highly toxic heavy metals (Cr^{6+}) which cause water pollution (Sing, 1994), where a lot of (>170000 tons) chromium wastes are released to the surroundings (Kamaludeen et al., 2003). It causes health hazards since it can easily enter biological cell membranes (Chaudhary et al., 2003). Tanned skin-cut wastes (SCW) which are used to produce feeds and fertilizers, are the direct phenomenon of chromium toxicity (Rafiqullah et al., 2008). Hexavalent Chromium (Cr^{6+}) is 100–1000 times more poisonous compared to trivalent (Cr^{3+}) form (Gauglhofer and Bianchi, 1991). So, conversion of Cr^{6+} is one of the significant mechanisms for microorganisms that can be used for detoxification of chromium.

Lacking a single waste-water treatment facility, a notorious and substantial ruin of the environment is another fate of concern from such a pivotal industry to sustain a billion-dollar business. An excess of such chemicals in the water and soils is harmful for the health of the people crammed into the area (Sunder et al., 2010). The breakthrough toward the sustainable mitigation of this overwhelming problem is nothing but the installation of an appropriate effluent treatment plant in every industry in terms of efficiency, cost effectiveness, simplicity and more importantly it should be environment friendly. Due to lack of treatment plants and environment management schemes in most of the tanneries in our country, raw wastes are simply discharged into the environment, causing severe environmental and public health troubles in particular areas. Appropriate environmental management is needed (Hasnat et al., 2013) to overcome this hazardous issue and tannery productivity. Several microorganisms have developed detoxification and respiration mechanism using heavy metals and thus become resistant to it (Ezaka and Anyanwa, 2011). The isolation and characterization of heavy metal resistant bacteria is significant for its metal accumulation capability along with its resistance capacity. In our study the sampling sites are the three nearby surroundings of Madina tannery which is the largest tannery located at Jalalabad area, near Oxygen point in Chittagong, Bangladesh. It was established in 1983, and is renowned for manufacturing all kinds of export quality crust and refined leather. In the surroundings of Madina tannery, large municipal areas have been observed.

So, the present study aims to investigate the ability of natural inhabitant bacteria of tannery effluent in reducing and detoxifying of heavy metals (Pb, Cr and Cd) at privileged conditions, where objectives include – isolation of naturally occurring bacteria from tannery effluent, screening of top three isolates as the reducer of Pb, Cr and Cd, characterization of heavy metal resistance, identification of those bacteria up to genus and profiling of their plasmids as a fundamental research to ensure the basis of their resistance in order to use them for detoxification in an incorporated bioremediation scheme.

Materials and methods

Study area and collection of samples

Tannery effluent samples were collected from three agricultural and residential sites beside Madina tannery (Fig. 1D, Table 1), in labeled pre-sterilized bottles, and cold chain was maintained during shipment to the laboratory in the University of Chittagong. Collected samples were preserved at 4 °C before analysis and during experiments.

Primary screening of heavy metal resistant bacteria

For the selective screening of heavy metal resistant bacteria, 300 μg/mL of heavy metal (Lead) incorporated LB (Luria Bertani) agar plates (Peptone 10.00 g/L, yeast extract, 5.00 g/L, NaCl 5.00 g/L, dextrose anhydrate 10.00 g/L and agar 30.00 g/L: pH −7.00) were used and screened by standard pour plate method observed at 37 °C. After 24 h of incubation the plates were observed for any kind of development on the culture medium. After preliminary screening of effluent samples containing heavy metal degrading isolates, serial dilution was done as Azad et al. (2013) to isolate desired bacteria. Streak plate technique was followed during isolation. Control plates also prepared with LB media without including any heavy metal to make comparison. Colonies differing in morphological characteristics were selected, picked, purified and then preserved on different plates for further studies.

Multiple metal resistance capacity

All isolates (S1, S2, S3, S4 and S5) were separately grown on LB agar plates supplemented with Cd, Cr and Pb (300 μg/mL) at pH 7.0 and 37 °C for 24 h; whereas after incubation the resistance capacity of multiple heavy metals was assessed.

Relative effects of heavy metal consumption on microbial growth

The optimal growth conditions with reference to different amounts of three heavy metals were determined. The isolates were grown in a rotary shaker (Wise cube®, Korea) at 150 rpm and pH 7.0, while the temperature was 37 °C in LB broth medium supplemented with different types of heavy