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ARTICLE

Isolation and Characterization of Bacterium isolated from Bantala Tannery Solid Wastes

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ABSTRACT

Leather industries that uses the conventional chrome tanning process are subjected to high risk of contamination due to the emission of toxic Cr(VI) that poses a serious threat to the environment and human's wellbeing. The present study were made to isolate and characterize chromium tolerant bacteria in the samples collected from four different plots of Bantala Tannery, Kolkata, West Bengal, India. Pure chromium tolerant bacterial strains were isolated from the tannery sludge samples and their relative MIC (Minimum Inhibitory Concentration) were recorded at different concentrations of Cr (VI) salts to select the highest chromium tolerant bacterium. The selected bacterium was further taken for their growth studies followed by different cultural, morphological and molecular analysis (16S rDNA). The bacterial strain was further studied through SEM (Scanning Electron microscopy) and EDX (Energy Dispersive X-ray) spectroscopy which revealed that TW4 was a gram positive, rod shaped, endospore forming, pleomorphic bacterium with phylogenetic similarities with *Isoptericola sp.* and genebank accession number SUB1732465 TW4 KX640927.

1. Introduction

The process of production of leather has been a very important process since ancient times due to its high demand in daily life. The process involves several mechanical and chemical stages. Most of the tanneries release the untreated wastes in the environment. This leads to deposition of huge amount of solid wastes and wastewater containing toxic chromium salts used during tanning (Alam and Malik, 2008; Familec *et al*., 2011)[1][7]. The presence of high concentration of chromium leads to the adaptation of microorganisms that can develop the mechanism to withstand the metal and sustain in the hostile environment.

The leather complex which is 20 Kms from Kolkata, West Bengal, India on its south-eastern periphery, is a living hell.

The smell of the chemicals used to treat the leather

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often sickens one to nausea. The water canal gets choked with rotting animal hair, fat and the omnipresent plastic. The visible canal water often gets coloured with blood, dyes or chromium and even shines in grimy bubbles. Most of the tanneries are devoid of the infrastructure which may suitably treat the tannery plant effluents. The burning and boiling of shaving dust, flesh linings and trimmings are often used to serve as fertilizers and fish feeds. The chromium content in these can pollute surface water and also can leach down to contaminate ground water. The supply of water laden with chemicals and salts in the surrounding farming lands has devastatingly reduced paddy yield. (Bera, 2013; Banerjee *et al*, 2018)[4][3]There had been very scarce studies available regarding the microbial population of the area and their potential in improving the metal toxicity of the environment. It is of immense need to start a study on the potential bacteria of the place that can thrive in the hostile environment so that it may help in remediation of the wastes in near future. Thus the present study was carried out to elucidate the isolation and characterization of potential chromium tolerant bacteria from tannery effluent (Alam and Malik, 2008)[1].

2. Materials and methods

2.1 Isolation of bacterial strains

Bacterial strains resistant to Cr (VI) were isolated from solid tannery sludge sample collected fromCalcutta Leather Complex, Bantala, East Kolkata, West Bengal, Indiausing nutrient agar

(NA), (Hi Media, Mumbai, India) plates supplemented with Cr (VI) salt ($K_2Cr_2O_7$). Ten grams of soil sample was suspended in 90 ml sterile water and shaken vigorously for 10 min. A 0.1 ml aliquots of appropriate dilutions were plated on NA plates and incubated at 30 °C for 24 h. Individual bacterial colonies on NA plates which varied in shape and colour were picked up and purified by repeated sub culturing on the same medium. Sludge samples were also analyzed for the total chromium concentrations by atomic absorption spectrophotometer (Instrument: Varian AA240FS; Flame atomizer. Software: Work sheet Oriented AA software; Version 5.1 pro).

2.2 Identification of isolate

In the present study, a total of 16 bacterial isolates were isolated from the chromium contaminated solid sludge. Bacterial isolates were identified on the basis ofcultural characterization of the bacterium following the *Bergey's Manual of Systematic Bacteriology* and morphological characteristicsthat included negative staining, gram staining and spore staining following the established methods (Aneja,2003; Ghori *et al*., 2011)[2][8].Out of the 16 isolates, the isolate TW4 was specially chosen due to its simultaneous high resistance towards chromium. This isolate was further identified by 16S rDNA analysis using the primers 8F: (5′AGA GTT TGA TCC TGG CTC AG 3′) and 1492r (5′-GGT TAC CTT GTT ACG ACTT-3′) as *Isoptericola* sp. TW4. The Gen Bank accession number of 16S rDNA sequence of the isolate is SUB1732465 TW4 KX640927 (Shakoori *et al*., 2010; Shekhar*et al*., 2014)[10][11].

2.3 Minimum Inhibitory Concentrations of Chromium

Minimum inhibitory concentration (MIC) of chromium against the test isolates weredetermined by the plate dilution method. The metal Cr^{6+}were used as $K_2Cr_2O_7$ (Hi Media, Mumbai, India), respectively, in increasing concentrations ranging from 50ppm to 250ppm were added to sterilized NA and poured into plates which were then spot (10 µl) inoculated aseptically with exponentially growing culture of *Isoptericola* sp. TW4. The plates were incubated at 37 °C for 24 hrs. The lowest concentration of the metal at which no growth occurred was considered as MIC.

2.4 Growth Studies

Growth of the *Isoptericola* sp. was determined by taking about 5ml of 24hrsbroth culture which was aseptically transferred to a fresh broth culture amended with 50ppm-$K_2Cr_2O_7$.Initial optical density (OD) at 600nm wavelength was recorded and the inoculated culture flask was placed in the shaker (120rpm) at 30ºC for 12hrs. After 1h of incubation, 5ml of the culture was aseptically transferred to a cuvette and the optical density of the sample was recorded at 600nm. A control was studied simultaneously with the sample culture. OD values were recorded at 1h interval and a growth curve was obtained using the observed OD of the control culture and the sample culture (Chaturvedi, 2011)[5].

2.5 Scanning Electron Microscopy and Energy Dispersive X-ray Analysis

The sample for the SEM and EDX study was prepared by taking 1mL of bacterial broth which was centrifuged at 12,000rpm. The pellet was treated with 4% gluteraldehyde (in Na- phosphate, pH - 7.2) after buffer wash and kept overnight. Dehydrolysis of sample was followed by different volumes of ethanol starting from 50%, 70%, 90%, 100%.SEM and EDX stub was prepared by applying the adhesive tape and fixing of the glass slides smeared with the treated bacterial cultures. Slides were then screened under a Scanning Electron Microscope and images were used to generate the EDX report (Narayani,2012)[9].

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3. Results and Discussion

The total chromium concentration was found to be 21.43 mg/gm of sludge sample. The *Isoptericola* sp. TW4 was isolated from solid tannery sludge heavily contaminated with chromium. The bacterial colony had white colouration. The different staining analysis showed the bacterium as rod shaped (Plate 2), gram positive (Plate 3) and endospore forming bacterium (Plate 4).The partial 16S rDNA sequence of the bacterial isolate was compared with the sequences in GenBank database by BLAST-N algorithm to identify sequences with high degree of similarity. The gel, when UV transilluminated, revealed a very bright, thick band of PCR product which was about 1.5 kb in size (Plate 5).Amplification of 16s rDNA yielded a 1475 bp product. The isolate TW4 showed 99% sequence similarity with *Isoptericola sp.* (Fig. 1). So, from 16s rDNA partial sequence data along with cultural features and morphological characteristics, the TW4 isolate could be identified as belonging to the genus *Isoptericola*. The bacterium is classified as belonging to the phylum Bacteroidetes, the class Flavobacteria, the order Flavobacteriale and the family Flavobacteriaceae. This genus was first described by .

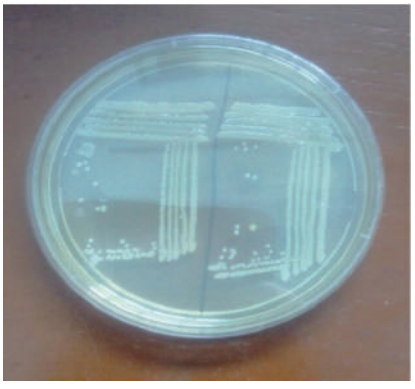

Plate 1. Establishment of pure cultures on NA plates amended with 50ppm $K_2Cr_2O_7$

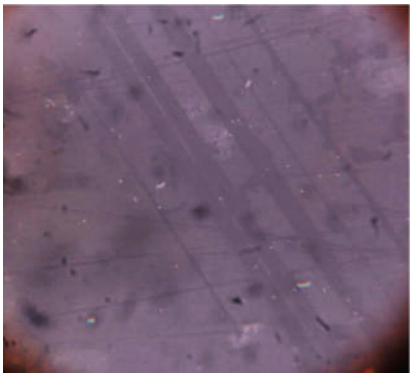

Plate2. Negative staining (1000X)

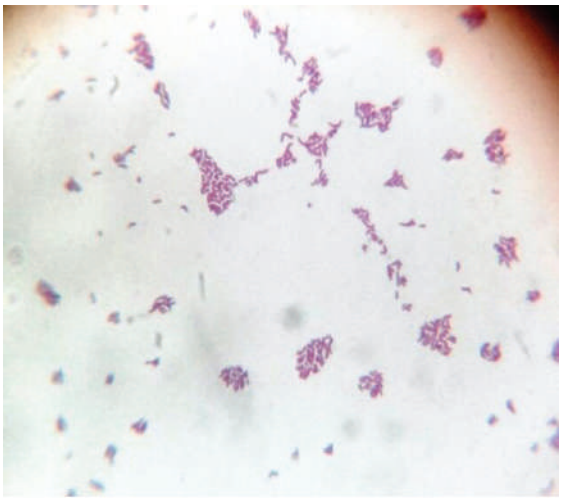

Plate 3. Gram staining (1000X)

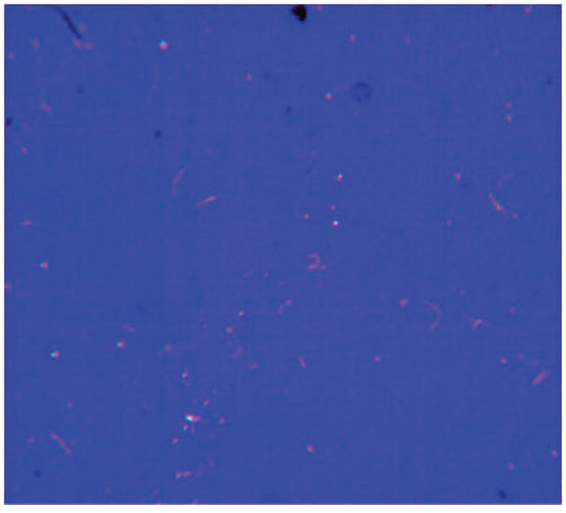

Plate 4. Endospore staining(1000X)

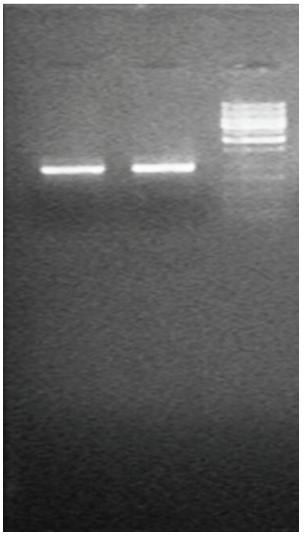

Plate 5. 16S rDNA PCR amplified product

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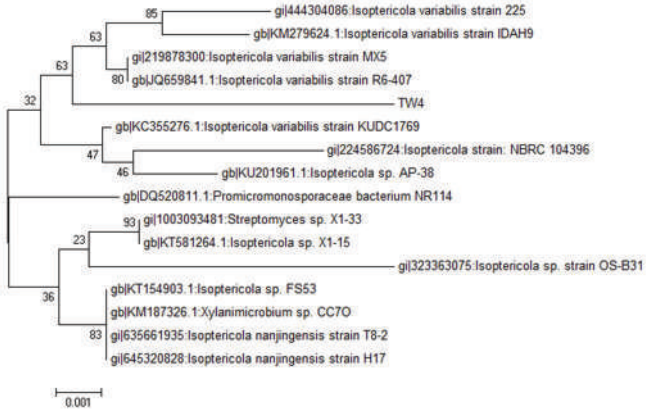

Figure 1. Phylogenetic analysis of TW4

Isoptericola sp. TW4 was found to show highest resistance against 250ppm of $K_2Cr_2O_7$. High level of chromium resistance in different bacteria was reported previously by several workers (Alam and Malik, 2008)[1]. Reports on chromium resistance by the genera *Isoptericola* are very rare. The bacterial growth curve clearly showed a distinctive change in growth between the bacteria under control condition and chromium stress condition (Fig. 2). The control bacteria showed a steep rise in its growth while the bacteria treated with $K_2Cr_2O_7$ showed a drastic reduction in growth.

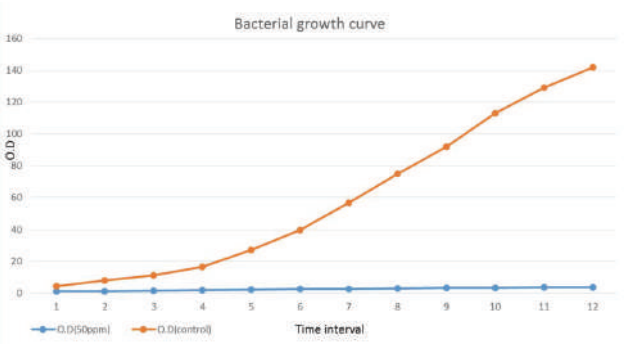

Figure 2. Growth studies of TW4

The SEM report of the isolated organism TW4 clearly revealed a drastic morphological change (from rods to spherical) when the organism was treated with 100ppm Chromium(VI)salts which is a typical characteristic of pleomorphic bacteria (Plate6; Plate7). Comparative EDX analysis between the control and the 100ppm chromium(VI) treated organisms clearly depicted that there were trace amounts of stress accumulation of chromium in the treated organisms (Plate8;Plate9) (Das *et al*.,2014)[6].

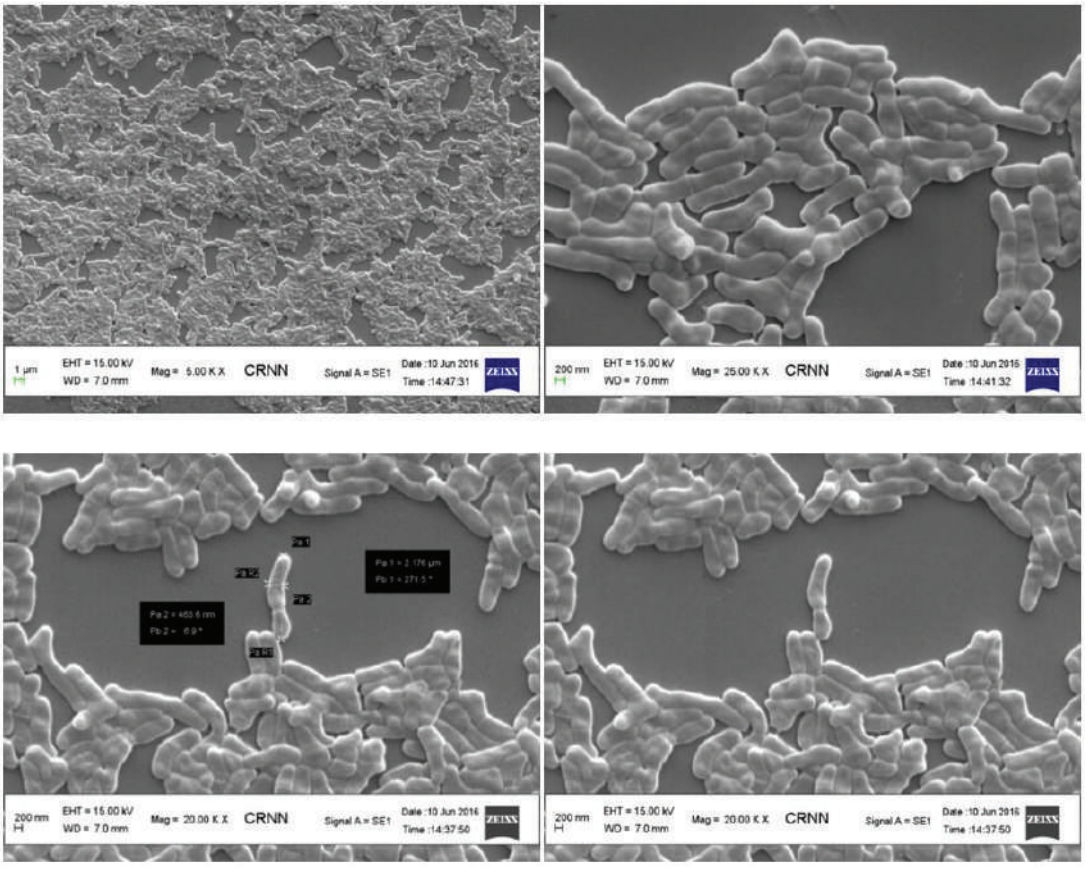

Plate 6. Control images of TW4 under SEM

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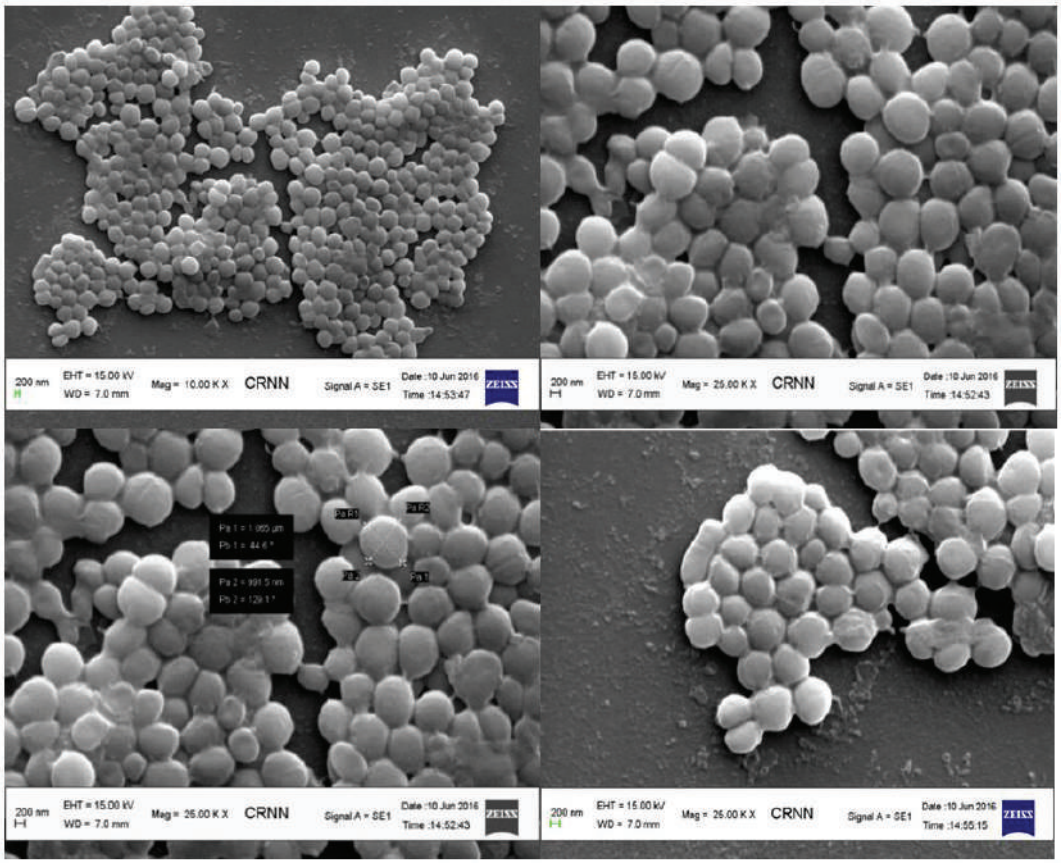
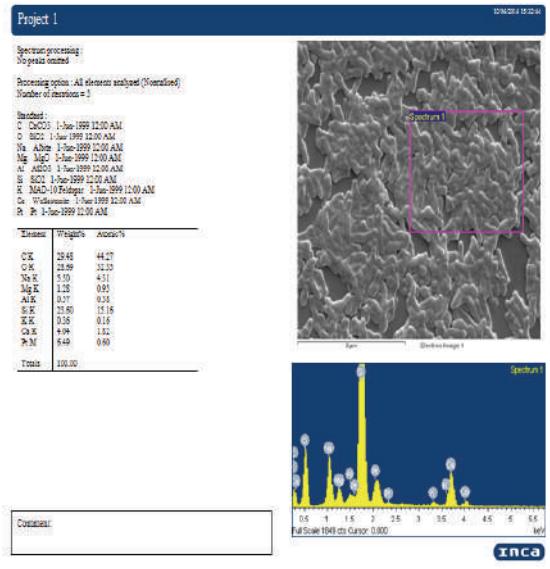


Plate 7. SEM images of TW4 at 100ppm of Cr (VI)



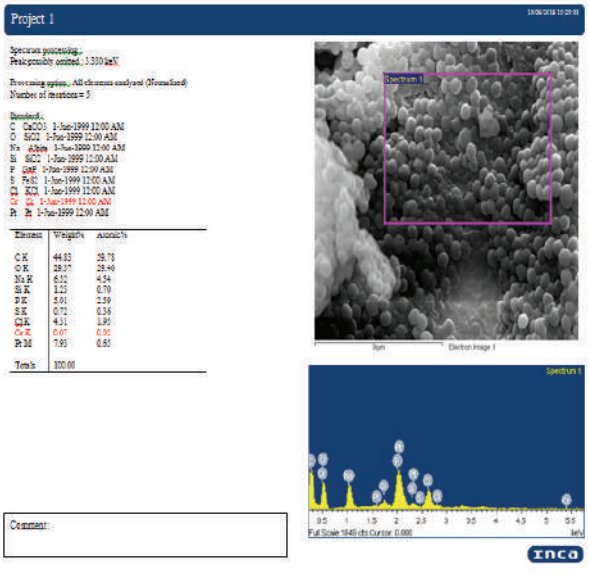


Plate 8. EDX analysis of control sample of TW4

Plate 9. EDX analysis of sample TW4 treated with 100ppm of Cr (VI)

4. Conclusion

Till date, not much is known about the Isoptericola genus in general. Therefore, these results add a clue as to their function in the environment especially with respect to

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their morphological changes related to chromium stress. These results demonstrate that *Isoptericola* sp. TW4 was resistant to high level of chromiumand showed a rapid morphological change (from rod to coccus) under high stress of chromium. The bacterium also showed stress accumulation of trace amount of chromium. Therefore, the *Isoptericola* sp. TW4 used in this study could be exploited for remediation of soil and waste streams contaminated with chromium.

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